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COARCTATION OF THE AORTA AND THE AORTIC ISTHMUSES

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COARCTATION of the aorta, the local narrowing of the aortic arch just as it turns downward to become the descending aorta, has been recognized for many years, and the accompanying compensatory enlargement of the collateral circulation, on the completeness of which in extreme cases continuation of life depends, has been known for a long time. The realization that coarctation is of two main types, however, is due to Bonnet[1], who, in a series of articles in the *Revue de Medicine*, in 1903, made a distinction between a newborn type and an adult type, so called from the period of life in which each type is most frequently encountered. These he called type I and type II respectively, and as lesser divisions he mentioned type III, in which the adult type is found in infants, or the newborn type in adults, and type IV, including all other irregular forms. For the two main types he indicated clearly the distinguishing characteristics; yet after nearly half a century certain recent articles on the subject of coarctation show that the distinction between the two main types is not generally understood. The purpose of the present paper is to reemphasize the characteristics of the two types and to reexamine from an embryologic point of view the common theories as to the causation of type I of this anomaly.

In the adult type, as described by Bonnet, the stenosis is always in the immediate vicinity of the ductus arteriosus or ligamentum arteriosum, "rarely just above, usually below, always touching it." The lesion itself is an abrupt strangling of the vessel, "as if the aorta had been constricted by a ligature . . . sometimes the aorta seemed bound by a ribbon instead of by a thread"; almost always the constriction is much more pronounced on the convex wall (fig. 1 *a* and *b*). Bonnet ascribed this constriction to the atrophy of the ductus, believing that either the shrinkage following its degeneration exerted simple traction on the aortic wall or the degenerative process acting in the ductus spread around the aortic wall as a narrow band and

From the Children's Hospital,

1. Bonnet, L. M.: Rev. de med., Paris **23**:255, 355, 419 and 481, 1903.

produced a ring-shaped local contraction of the latter vessel. The latter concept fits better with present knowledge of the mechanism of the normal closure of the ductus, which, according to Schaeffer[2], entails a rapid overgrowth of the subendothelial tissue, which takes the form of several bulging cushions that increase in thickness until the lumen is obliterated, an accompanying growth of elastic tissue fibers within and just peripheral to the cushions, and a final fibrous degeneration of all the tissues of the vessel wall with resulting contraction and scar formation. The whole process may occupy six or eight weeks. This or some similar sequence invading the contiguous aorta in ring form might well explain the adult type of coarctation. Within the lumen of the aorta the ring-shaped constriction may take the form of a thin iris diaphragm either closed or with a larger or smaller central perforation, which is rather characteristic of this type, frequently accompanied by short longitudinal ridges of similar material projecting from the wall just above the diaphragm in such a way that they might form valves to close the perforation. Both ridges and folds probably represent the remains of the endothlial cushions. If the degenerative process in the aorta follows that in the ductus, the slowness of the action may allow ample time for the establishment of the collateral circulation and explain the common discovery of this type in adults.

As the explanation of coarctation of the newborn type, now more commonly called the congenital or fetal type, Bonnet accepted the already current theory of the persistence, after birth, of the aortic isthmus, frequently present in the fetus, but he accepted it only with restrictions. The aortic isthmus has long been known as that segment of the aorta between the origin of the left subclavian artery and the entrance of the ductus arteriosus. It is a potentially stagnant section of the aorta in the fetus, for as long as placental circulation persists the arterial blood, through the foramen ovale and the left ventricle, traverses the aortic arch to reach the head and the arms, and the equally arterial blood going to the lower part of the body and the legs passes through the open ductus. At birth the right ventricle, though not so large as the left, has an equal or even greater musculature. Partially deprived of current, the isthmus remains small, for the enlargement of fetal arteries directly depends on the increase of the contained blood stream. After the change of circulation accompanying birth the isthmus normally expands within a short time to full size; failure to expand results in coarctation. Bonnet considered this failure as due to simple "arrest of development," a term used to indicate that during development a certain structure remains in its

2. Schaeffer, J. P.: J. Exper. Med. **19**:129, 1914.

embryonic or fetal form, lacking the developmental energy to complete its normal growth. He also noted as a possible cause of the fetal isthmus and its commutation to infantile coarctation the frequently concomitant absence of the interventricular septum, which would lessen the circulation through the aortic arch. He saw, however, that this explanation could not apply to those cases of coarctation in which the stenosis was severe, even to the point of closure of the lumen. He speculated that the arrest of development in these extreme cases might have occurred at a much earlier period in embryonic life, or that the embryonic aorta might have been involved in the early atrophy of the disputed "fifth" arch.

The fifth aortic arch (arch V), situated between the embryonic arch IV (the adult aortic arch) and the pulmonary arch VI (represented by the ductus arteriosus), though present as a true arch in lower vertebrates with five or more pairs of branchial pouches, is rudimentary in mammals, which have only four pairs of pouches. It can frequently be found in human embryos of six weeks as a tiny vessel joining the fourth as the pulmonary arches, or making a loop from one or the other, or running as a branch from the middle of the pulmonary arch to the dorsal aorta between the two arches; it is rarely a true arch from ventral to dorsal aorta. Normally it lasts for only a few hours or days, and its very existence was long disputed by anatomists; yet a single case reported in the autopsy records of the Children's Hospital[3] suggests that the fifth arch may, very exceptionally, not only persist much longer than usually supposed but even cause coarctation of the aorta. As the records reads, "a band or valve-like structure which must have occluded approximately half the lumen" is attached to the superior wall of the aorta at a point 2 mm. beyond the left subclavian artery, and "opposite this band is a pucker of the intima suggesting the focus of the origin of another blood vessel," but no other sign of a lost vessel was seen in the wall or on its surface. The patent ductus is present just below. This case in all probability indicates the prolonged persistence of the fifth arch and its complete disappearance at some time shortly before birth, the partial diaphragm and the opposing dimple remaining as the only signs of its presence and degeneration. The coarctation, however, is of the adult type, not of the infantile, as postulated by Bonnet.

In the classic description of the aortic isthmus and of the derived congenital coarctation the narrowing is located between the root of the left subclavian artery and the distal attachment of the ductus arteriosus, and on this premise is based the theory of the division of the blood streams to head and arms and to body and legs, with

3. The case is reported in the autopsy records as No. A-39-67.

minimal current in the intervening segment of the arch. In a few recorded cases, however, the coarctation is described as "involving the subclavian"[4] or "above the subclavian,"[5] the upper limit of the narrowing then being the left common carotid artery (fig. 1). The subclavian artery has even been reported as included in or below the site of coarctation of the adult type[6]. Moreover, I once noted marked narrowing at the isthmus in a fetal chick two days before

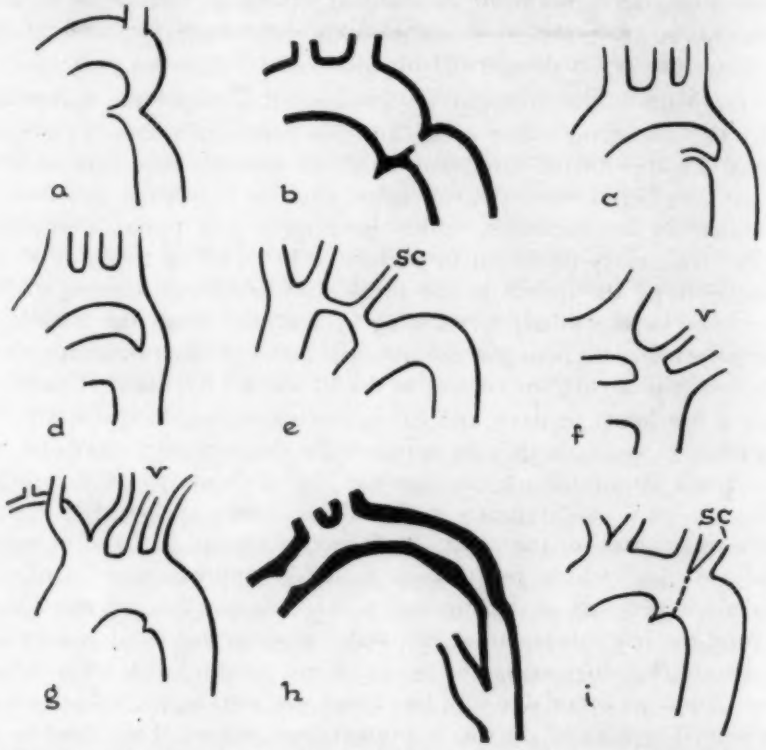

FIG. 1.—Various forms of coarctation: (*a* and *b*) type II (Bonnet); (*c* and *d*) type I, (Bonnet); (*e*) type IV, case observed at Children's Hospital; (*f*) type IV (Bonnet); (*g*) normal variation of branches of the aortic arch; (*h*) type seen in chick 21 days old; *i*, coarctation seen in man 26 years old, type IV (Parker and Day[6]).

hatching, although in the bird long before this time both subclavian arteries have transferred their roots, by the anastomosis of branches, to the two brachiocephalic trunks which arise from the ventral end of the fourth arch. The variations in the position of the human left

4. Schwartz, S. P., and Greene, D.: Am. Heart. J. **23**:99, 1942.

5. Bailey, R. H., and Holoubek, J. L.: Brit. Heart J. **2**:208, 1940.

6. Parker, R. L., and Day, T. J.: Am. Heart J. **15**:739, 1938.

subclavian artery become understandable when one considers that in the embryo this vessel is an outgrowth or continuation of the seventh intersegmental artery. The small intersegmental vessels, the first of which sprouts from the dorsal aorta on each side opposite the entrance of the fourth aortic arch, produce the vertebral artery by a longitudinal anastomosis of their tips; the upper six then degenerate. The seventh, arising far below the ductus, becomes the root of the subclavian artery, which bears the vertebral as its first branch (fig. 2). From this low position the subclavian artery migrates cranially as the heart moves caudally, until during fetal life it passes the ductus arteriosus and moves onto the aortic arch. By arrest of development it may not complete this upward journey and may remain at a lower level, somewhere along the descending aorta. It seems clear, then, that the normal position of the left subclavian artery is not a neces-

FIG. 2.—Diagrams of (*a*) the primary symmetric plan of the aortic arch system and (*b*) the normal plan after the loss of certain members.

sary element in the congenital type of coarctation or of the preceding isthmus.

The left vertebral artery also may migrate independently. Its root may be derived from the sixth intersegmental branch and hence be unconnected with the subclavian artery, or it may migrate along the subclavian to and up the dorsal aorta, with its own root. Rarely it may, as a recognized variation, arise from the normal arch as a separate branch between the left common carotid and the subclavian arteries (fig. 1*g*), and in congenital coarctation it has been noted arising from the center of the constriction, below which is the subclavian artery (fig. 1*f*).

Bonnet, in his various considerations, did not mention an earlier explanation of the aortic isthmus given by Stahel[7] in 1886. This author, writing rather of the aortic spindle, the enlargement which

7. Stahel, H.: Arch. f Anat. u. Entwcklngsgesch., 1886, p. 43.

immediately follows the isthmus, than of the isthmus itself, attributed its presence to the sharp curve of the aorta at this point, like the kink in a sharply bent rubber tube, and maintained that the spindle occurs only when the arch is flat and the angle abrupt, not when the arch is gently rounded throughout — the "flat" arch versus the "high" arch type — a theory still mentioned in certain textbooks. Moreover, Stahel found the spindle not only in the aorta but also in the first portion of the right subclavian artery, a condition not usually mentioned in the books, but important in the present investigation, for these two regions are embryologically strictly comparable, as can be seen by examination of the well known diagrams of the embryonic aortic arches and the changes they undergo in producing the normal fetal pattern (fig. 2). Of the early symmetric system (diagram *a* in fig. 2), certain portions atrophy in the embryonic period, as shown in the second diagram (*b*); the paired first and second arches, the dorsal portion of the right pulmonary arch, the caudal portion of the right dorsal aorta from the root of the right subclavian artery to the union of the two dorsal aortas, and, most important in the present consideration, the two dorsal aortas between the third and fourth arches. This last mentioned atrophy of the segments of the paired dorsal aortas takes place between the sixth and the eighth week. The atrophy of the dorsal end of the left pulmonary arch accompanies birth. When the left fourth arch assumes dominance and becomes the permanent aortic arch, the remaining vessels gradually rearrange themselves. The right fourth arch, now the first branch of its large twin, becomes the innominate artery and, beyond, the common carotid branch (representing part of the ventral aorta) continues as the first portion of the right subclavian artery; but, although displaced and reduced in importance, this portion of the right subclavian artery is embryologically symmetric with the dorsal portion of the aortic arch.

Reasoning that, since the adult type of coarctation is in all probability directly connected with the obliteration of the ductus, the two symmetric isthmuses, one of which may produce congenital coarctation, might also be connected with the obliteration of other vessels, and realizing that the lost sections of the two dorsal aortas between the third and fourth arches were in precisely the position to serve as the causative agents, I examined the sectioned embryos of the Harvard Embryological Collection for signs of narrowing at these two points at or after the six to eight week period. The human embryos were disappointing; no actual narrowing of the specified vessels was seen. It is often worth while, however, to look at other mammalian forms. The white rat is especially well represented in this collection by closely graded stages, and in the rat at the points in question,

i.e., the dorsal ends of the two embryonic fourth arches, the postulated constrictions were abundantly present.

The condition found in the rat embryo of seven weeks is given in the composite reconstruction (fig. 3) of the aortic arch systems of 3 litter mates of this age cut, one each, in the transverse, sagittal and frontal planes, showing the aortic arch, with its two branches, the innominate (right fourth arch) and left common carotid arteries, and the right pulmonary arch or ductus arteriosus. The two subclavian arteries are in a low position, but still slightly advanced, as

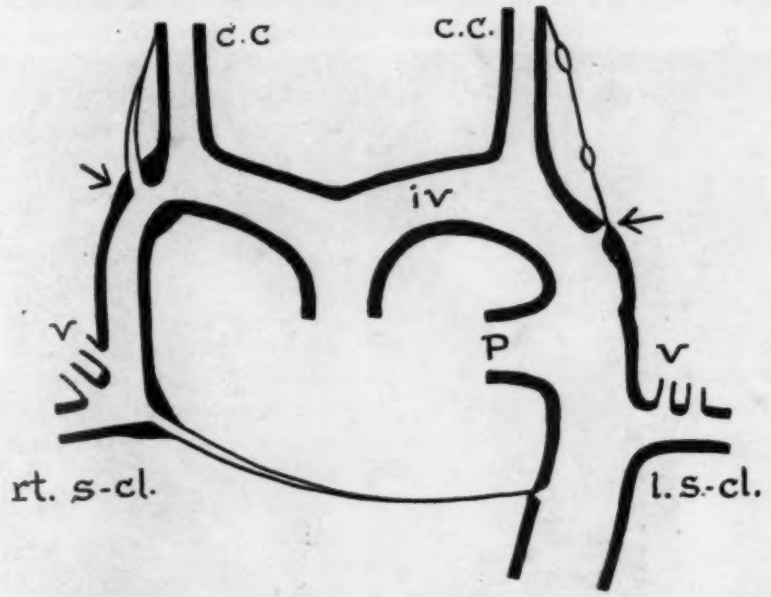

FIG. 3.—A composite reconstruction of the aortic arch system of rat embryos of 7 weeks (compare fig. 2 *b*). Arrows point to isthmuses and indicate the position of the sections shown in figure 4.

can be seen by the length of the lower part of the right dorsal aorta. This degenerating portion, and also the two dorsal aortas between arches III and IV are presented by fibrous cords with occasional vesicles or narrow, still open sections. Below, at the former junction of the two dorsal aortas, the remnant of right aorta shows a simple funnel-shaped opening through the aortic wall, but at the other end of the remnant the wall of the shortened right aorta is somewhat thickened. At the junction of the atrophic dorsal aortic segments and the two fourth arches the thickness of the arch walls is much increased, and also, especially on the right side, the thickness completely encircles the vessel and constricts its lumen, producing a definite isthmus.

It seems difficult to correlate this early type of isthmus with the later type of adult coarctation, the ultimate cause of which is the increase of subendothelial tissue (endothelial cushions) in the closing ductus. The aorta at seven weeks has no organized histologic structure of its walls, the endothelium resting apparently directly on the dense mesenchyma beneath, which forms its sole support. However, when closely examined the more highly magnified photomicrographs (fig. 4) reveal the lifting or looseness of the endothelium in certain definite areas — at the mouth of the degenerating left dorsal

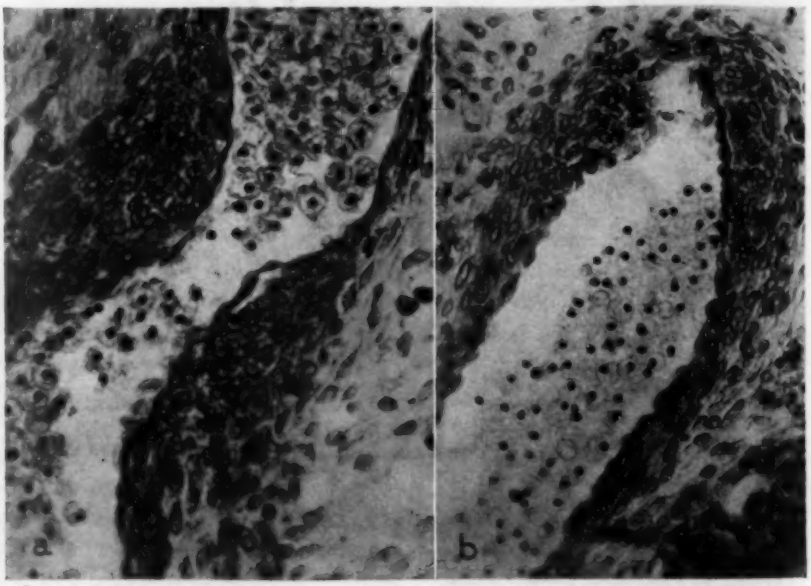

FIG. 4.—(*a*) Sagittal section through the right isthmus: The common carotid artery is seen above; the right subclavian artery, below. (*b*) Transverse section through the left isthmus at the entrance of the obliterating dorsal aortic section (top): below, aortic arch (thin-walled); above, isthmus (thick walls).

aorta (*b*) and more strikingly at the narrowest point of the isthmus on the right side (*a*). As all those familiar with microscopic slides are aware, the lifting of vessel endothelium throughout the slide is a common finding and is usually due to faulty fixation of the material, but when it occurs at only a few isolated sites in otherwise faultless sections, and occurs, moreover, at the same sites in adjacent sections in one series and in other embryos of the same age (on reviewing the human embryos with this in mind I have found the same loosened endothelium at the mouths of many dying vessels), the phenomenon becomes significant. It may indicate the local increase of an invisibly

narrow subendothelial sheet in the form of miniature endothelial cushions, imitating those in the closing ductus. In the isthmus shown in the photomicrograph (fig. 4 *a*) the miniature cushion forms a ring encircling the lumen and (in other sections of the series) continuous with or perhaps better extending or spreading out from the mouth of the degenerating dorsal aorta above. If this supposition is correct, if the cushions actually do extend from dying aorta to larger vessel, the primary steps of closure duplicate exactly, but minutely, those encountered in the adult type of coarctation.

According to the usual teaching, the portion of the right dorsal aorta between the right fourth arch and the subclavian branch in its embryonic position (compare fig 3) is shortened during further growth of the embryo and incorporated in the first portion of the right adult subclavian artery. It is more probable that the right subclavian artery undergoes the same migration as that of the left side, to the distal end of the isthmus and that the right vertebral artery also carries out its independent migration to a further point beyond the isthmus. But on the right the migration of the vertebral branch is almost constant, not infrequent as on the left, and is often accompanied by a similar migration of the neighboring branch of the subclavian artery, the thyrocervical axis. This would account for the common position of the subclavian isthmus distal to the vertebral branch, often also distal to the axis. The portion of the right dorsal aorta traversed by these migrations either completely degenerates or "not uncommonly," according to Patten,[8] remains in the adult as a small aberrant branch from the first portion of the right subclavian artery running behind the esophagus to join the descending aorta opposite the third thoracic vertebra.

On the right side the constrictive processes which cause the isthmus soon yield to the counterpressure of the increasingly more powerful force of the blood stream until at birth the isthmus is rarely noticeable. On the left the aortic isthmus lies in the "stagnant" section of the descending aorta, and, according to the volume of the passing stream (for the term "stagnant" is only relative), may remain as a fairly open isthmus and expand fully at birth, or may become increasingly narrow or even close completely, unable to open at birth in spite of the suddenly increased blood stream, the closure resulting in the congenital type of coarctation. The coarctation is due to the extreme stagnation of this section of the aorta acting on the already present isthmus; the isthmus itself depends on the normal degeneration, at about seven weeks, of the dorsal aorta between the third and fourth aortic arches.

8. Patten, B. M., in Morris, H.: Morris' Human Anatomy, edited by J. P. Schaeffer, Philadelphia, The Blakiston Company, 1942.

SUMMARY

The distinguishing characteristics of the two main types of coarctation, the congenital or fetal type I and the adult type II of Bonnet, are reemphasized. Many of the variations of both types (called collectively type IV) are shown to depend on the extent of the normal cranial migration of the left subclavian artery, or less commonly on that of the vertebral artery.

The adult type of coarctation is due to a fault of development in which the processes of closure of the ductus arteriosus normally occurring at birth extend abnormally to the aorta. The congenital type of coarctation is connected with the presence of the aortic isthmus. This isthmus and its counterpart on the right side, the less well recognized right subclavian isthmus, also are connected with the normal closure and degeneration of paired embryonic vessels, the segments of the two dorsal aortas between the third and fourth arches, lost during the rearrangement of the primarily symmetric aortic arch system at six to eight weeks. The subclavian isthmus is expanded by the constantly increasing volume of blood flowing through the growing artery. The aortic isthmus is located in the relatively stagnant section between the aortic arch and the pulmonary artery; depending on the volume and the rate of the flow of the blood circulating through this section, the isthmus may expand gradually until birth and then rapidly, or, with minimal volume, it may continue closing even to obliteration, the closure resulting in aortic coarctation or atresia.

PATHOLOGIC REACTIONS IN THE LIVERS AND KIDNEYS OF DOGS FED ALCOHOL WHILE MAINTAINED ON A HIGH PROTEIN DIET

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DESPITE the number of studies devoted to determining the role of alcohol in the causation of hepatic cirrhosis, a clearcut conclusion has not yet been reached. The inability to determine with finality whether or not alcohol per se induces hepatic damage and fibrosis can be largely attributed to disturbances of feeding frequently encountered in experimental animals and human subjects consuming large quantities of alcohol. Since malnutrition alone has been shown to induce hepatic injury, it is difficult to determine whether the hepatic disease occurring in association with experimental or clinical alcoholism results from the toxic effects of alcohol on healthy liver cells or from the increased susceptibility to alcoholism of liver cells previously injured by malnutrition.

Lillie and his associates[1] suggested that ". . . it would appear that alcohol gives an additional insult to liver tissue injured by a dietary deficiency." This opinion was subsequently corroborated by the more carefully controlled paired-feeding experiments carried out by Lowry and co-workers[2] in the same laboratory. This second study led to a more definite opinion, namely, "Alcohol increases the severity of the liver cirrhosis produced in rats with a deficient diet." In recent months Ashworth[3], on the basis of short term paired-feeding experiments in rats, has concluded that alcohol can be toxic to the liver, even when an adequate diet is fed.

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1. Lillie, R. D.: Daft, F. S., and Sebrell, W. H.: Pub. Health Rep. **56**:1255, 1941.

2. Lowry, J. V.; Ashburn, L. L.; Daft, F. S., and Sebrell, W. H.: Quart. J. Stud. on Alcohol **3**:168, 1942.

3. Ashworth, C. T.: Proc. Soc. Exper. Biol. & Med. **66**:382, 1947.

Several years ago an attempt was made in this laboratory to determine the effects of alcohol on the livers of dogs. In our first study it was found that severe and extensive disease of the liver, including cirrhosis developed in dogs maintained on a high fat diet and given large amounts of alcohol.[4] However, subsequent investigations revealed that an equivalent degree of damage of the liver can be produced in dogs by the feeding of the high fat diet alone, without the simultaneous administration of alcohol.

In the present study dogs were fed a high protein diet, adequate in all respects and known to be capable of maintaining a normal hepatic structure as seen in control animals. While the subjects were maintained on this high protein diet, large doses of alcohol were administered. As was to be expected, erratic feeding habits developed in the majority of the animals receiving alcohol, and food intake could be maintained only by forced feeding.

Despite every effort on our part to distinguish the effects of alcohol from those of erratic consumption of food in the livers of these animals (table 1), we feel incapable of doing so. The objects of this report, therefore, are to draw attention to the peculiar lesions encountered in the livers and the kidneys of these animals and to discuss their genesis and cause.

EXPERIMENTAL PROCEDURE

All dogs used in this study received once daily, at 8 a.m., a diet consisting of 30 Gm. of lean meat per kilogram of body weight, 2 Gm. of bone ash, 2 gm. of Cowgill's salt mixture,[5] 3 cc. of "sardilene"[6] and 5 cc. of "galen B."[7] Just before the meal and again at 4 p.m. the amounts of 22.5 per cent alcohol recorded in table 1 were administered by stomach tube. These amounts of alcohol were usually sufficient to produce a stupor, and when tolerance increased so that this no longer occurred, greater amounts of alcohol were given to insure stupor. Careful records were kept of the response and behavior of the dogs.

At autopsy several blocks of tissue were removed from the liver and the kidney for histologic examination. The liver was analyzed for total fatty acids by a method described elsewhere.[8]

RESULTS

The pathologic reactions encountered in the livers of these animals were fatty change (figs. 1, 3, 4 and 6), hemorrhagic necrosis (fig. 2), and fibrosis (figs. 3-6).

In the 3 animals examined at the end of nine and ten weeks of the experimen-

4 Connor, C. L., and Chaikoff, I. L.: Proc. Soc. Exper. Biol. & Med. **39**:356, 1939.

5. Cogwill, G. R.: J. Biol. Chem. **56**:725, 1923.

6. Each cubic centimeter of "sardilene" (an aqueous extract of rice bran) contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A.

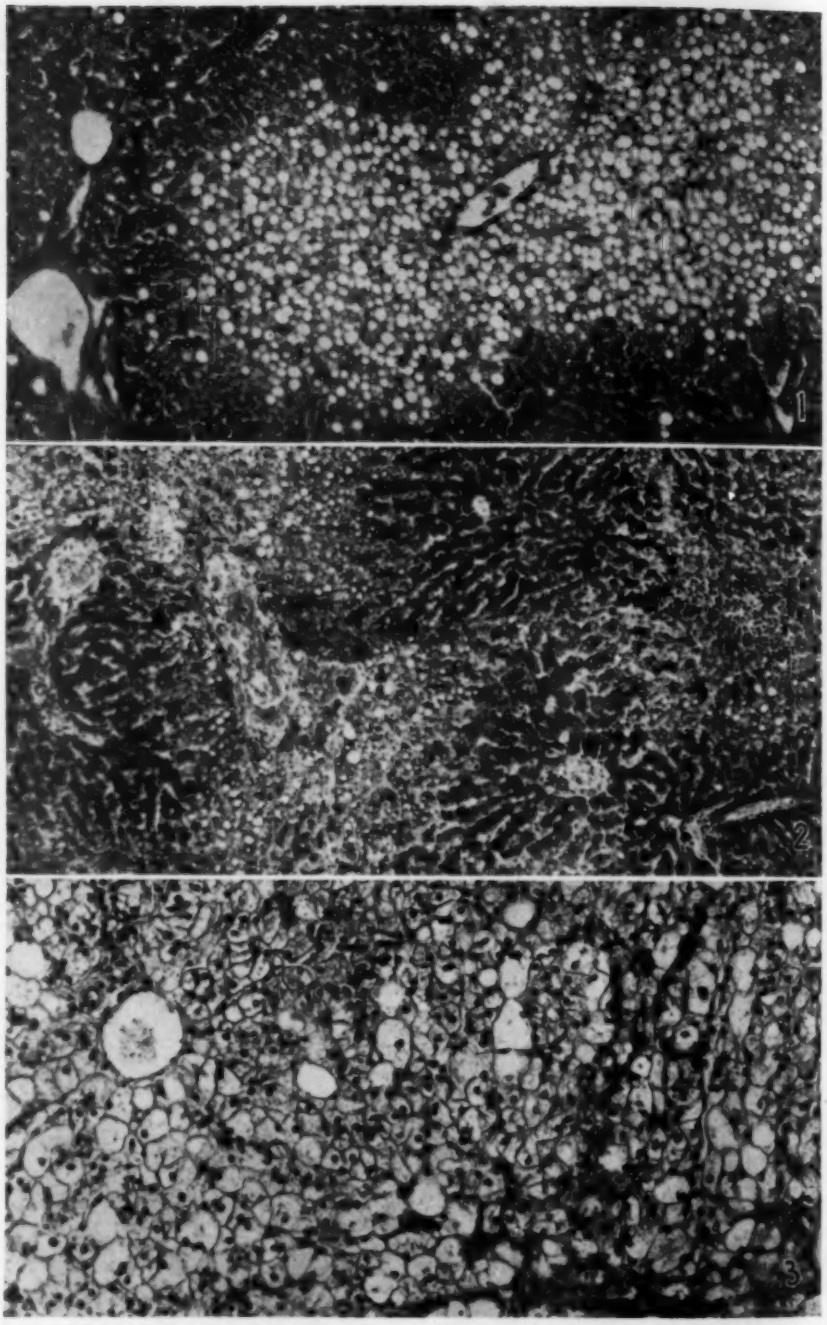
7. The vitamin content of "galen B" has been recorded elsewhere (Montgomery, M. L.; Entenman, C.; Chaikoff, I. L., and Nelson, C.: J. Biol. Chem. **137**:693, 1941.

8 Chaikoff, I. L., and Kaplan, A.: J. Biol. Chem. **160**:267, 1934.

TABLE 1—*Alcohol and Food Intake of Dogs*

Dog	Duration of Experiment, Weeks	Period of Administration of Alcohol, Weeks	Record of Administration of Alcohol*								Record of Food Intake
F31 ♂	9	7	Days	45							Ate well and retained food until the last 2 days, when it refused food. Died
			Cc. per day	180							
F32 ♂	130	93	Days	650							Ate all meals during first 3 months. During last 29 months ate about half the time. Force-fed last 13 days. Dog lost weight rapidly near the end. Died
			Cc. per day	170							
F51 ♂	10	8	Days	6	6	46					Ate well throughout. Died suddenly without prior weakening or sickness
			Cc. per day	120	160	210					
F52 ♀	72	61	Days	14	176	24	11	132	19	7	49	Ate poorly 1 month. Next 9 months ate all food. Ate about half the time for 3 months. Force-fed last 3 months. Became sick 2 days before end. Died
			Cc. per day	170	220	0	120	250	0	240	0	
F53 ♀	43	37	Days	7	7	107	139					Ate well for 3 months, then only about half the time for rest of experiment. Was not force-fed. Weakened during last week. Died
			Cc. per day	160	180	200	240					
F54 ♂	158	126	Days	5	36	202	20	621				Ate well for about 7 months. Ate about 1/3 the meals for 3 months. Ate well for 7 weeks and then was force-fed for 2 months; ate well 5 months; force-fed 6 weeks; ate well 9 months. Died
			Cc. per day	140	200	240	0	240				
F55 ♀	53	45	Days	36	20	9	37	31	90	3	90	Ate well for 2 months; ate 2/3 meals for 3 weeks; ate well for 3.5 months; ate about half meals for 5 months; vomited frequently; force-fed last 2 weeks. Weakened suddenly and died
			Cc. per day	160	220	200	120	170	280	120	240	
F56 ♀	30	26	Days	6	8	17	12	97	42			Ate well 2 months; ate 2/3 meals for 1 month; for remainder ate half meals. Hindlegs became paralyzed 2 days before death. Paralysis almost complete at time of death. Died
			Cc. per day	120	140	180	200	240	280			
F57 ♂	10	8	Days	7	8	22	20					Ate well for first 6 weeks and then ate poorly for balance of experiment. Died
			Cc. per day	120	140	180	220					
F58 ♂	130	112	Days	153	20	608						Ate well for 6 weeks; ate 3/4 meals for 2.5 months; well for 6 weeks; force-fed 2 months; ate well 23.5 months. Killed
			Cc. per day	240	0	240						
F60 ♀	104	90	Days	92	20	621						Ate well for 6 weeks; ate about 1/3 of the time for next month; ate well for 1 month; ate 2/3 meals 6 weeks; half meals for 2 weeks; force-fed 2 months; ate well 6 months; force-fed 5 weeks; ate well 9 months. Killed
			Cc. per day	240	0	240						
F61 ♀	59	51	Days	40	20	296						Ate well 6 weeks; force-fed 2 weeks; ate well 7 months; force-fed 2 months (vomited frequently); ate well 5 months; died suddenly
			Cc. per day	240	0	240						

*The amounts of 22.5 per cent alcohol U.S.P. recorded above were administered by stomach tube. Each number in the line designated "Days" refers to consecutive days (except for Saturdays and Sundays) during which alcohol was administered. Alcohol feedings were withheld when the dogs were in poor shape. As an example of the notation, take dog F51. This dog received daily for the first six days 120 cc. of 22.5 per cent alcohol. During a subsequent period of six consecutive days it received 160 cc. daily. The last or third period extended for forty-six consecutive days, during which time it received daily 210 cc. of 22.5 per cent alcohol.



Figures 1, 2 and 3

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tal regimen (see table 2: F31 at nine weeks, F51 and F57 at ten weeks) the livers manifested mild fatty change. The fat was localized for the most part in the central portions of the lobules and was present either as multiple droplets or as large globules in roughly equal amounts.

One of the animals studied at the end of ten weeks (F57) showed multiple areas of hemorrhagic necrosis with early replacement fibrosis, which involved about a third of the central portions of the affected lobules (fig. 2). This centrolobular hemorrhagic necrosis was associated with considerable simusoidal dilatation, the entire process apparently being acutely precipitated at the junction of the very fatty centrolobular cells and the nonfatty periportal liver tissue (fig. 2).

Another observation meriting record here is the finding in dog F51 of a greenish brown pigment material around the fat globules in the liver cells near the portal tract. This pigment, apparently a derivative or a precursor of bile, has been encountered by Gillman and Gillman[9] in the fatty livers of infants suffering from severe malnutrition. In the fresh specimen the pigment imparts to the liver a yellow color, whereas after the tissue has been fixed in solution of formaldehyde U.S.P. the material changes to a bright green, and as a result the portions of the liver containing it are clearly delineated. When excessive quantities of pigment are present, the entire liver turns emerald green. Attention is drawn to this intracellular bilelike pigment since in malnourished infants and in dogs it occurs in the absence of clinically or chemically detectable jaundice and is possibly indicative of some hitherto undescribed derangement of the metabolism of bile pigments associated with malnutrition and/or chronic alcholism.

The next group of 5 animals was examined at periods varying between thirty and seventy-two weeks after the commencement of the administrations of alcohol (dogs F52, F53, F56 and F61). Fairly severe fatty change, with single large globules of fat accumulating in the liver cells toward the central veins, was the most constant finding (fig. 1). However, within the fat-laden cells around the central vein of the liver in 4 dogs in this group there was a reaction characterized by thickening of the pericellular and intercellular reticulum and an inifltration of plasma and round cells, polymorhonuclear leukocytes, giant cells and fibroblasts; we have come to regard this reaction as early fibrosis (figs. 3 and 4). This reaction was most marked in dogs F56 and F53, killed at thirty and forty-three weeks respectively (figs. 3 and 4). It was least well developed, though present, in the animal examined at fifty-three weeks (F55), while it was totally absent in dog F61, studied at the fifty-ninth week of the experiment. In one of the animals examined at the seventy-second week (F52) this fibrotic reaction was not only extensive but also so well marked and associated with such a degree of structural distortion as to be classifiable as severe cirrhosis (figs. 5 and 6).

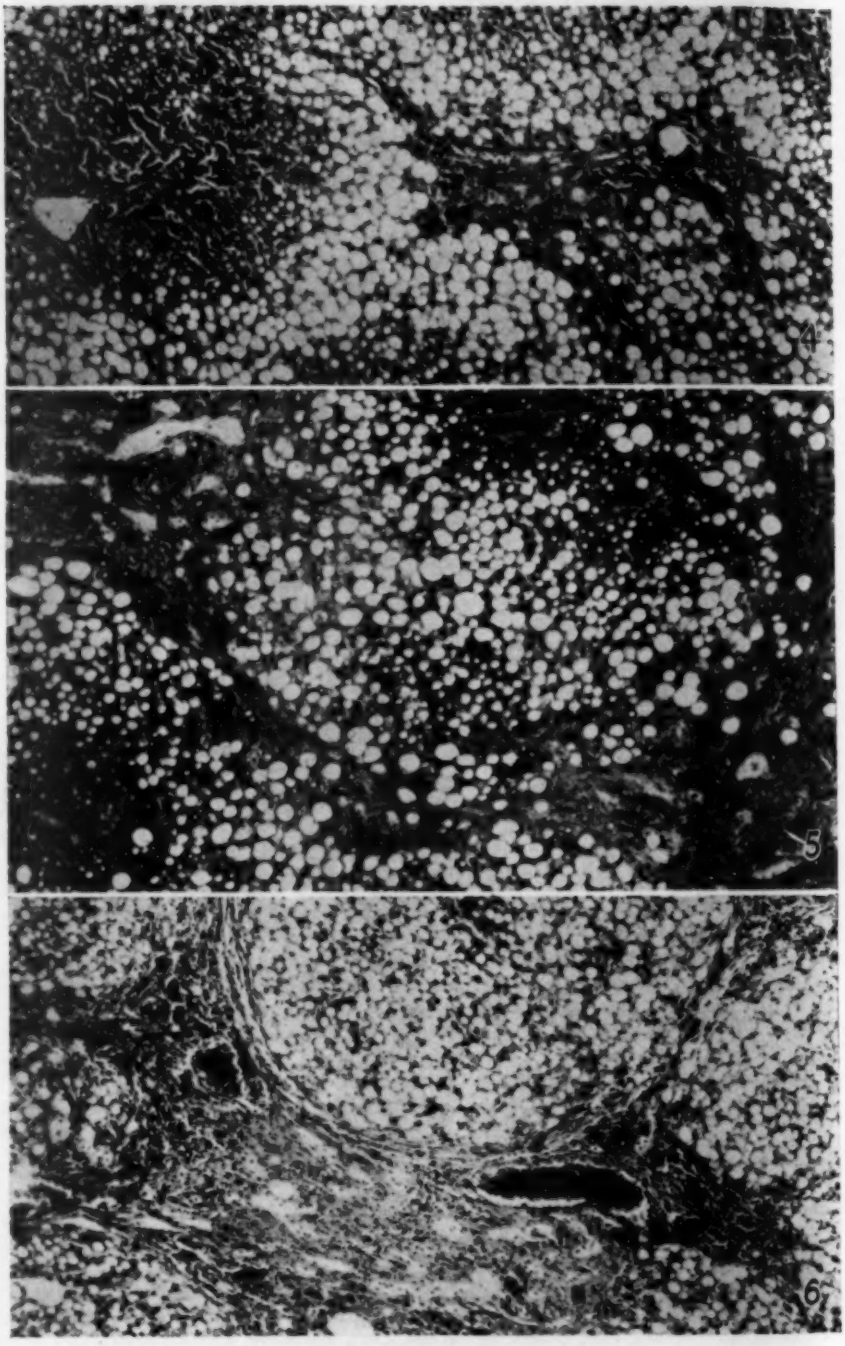
9. Gillman, J., and Gillman, T.: Monograph on Malnutrition in South African Negroes, to be published.

EXPLANATION OF FIGURES 1, 2 AND 3

Fig. 1 (dog F55).—In this severely fatty liver the predilection of the fatty change for the pericentral zone of the lobule may be seen, with a small zone of fat-free periportal cells. The central vein of this lobule is patent and free of infiltration and fibrotic changes. Hematoxylin and eosin; × 70.

Fig. 2 (dog F57).—Centrolobular hemorrhagic necrosis has been superimposed on a fatty change similar to that portrayed in figure 1. The pigment particles (dark spots) in the necrotic zone consist of hemosiderin. Note the absence of reaction in and around the portal tracts. Hematoxylin and eosin; × 65

Fig. 3 (dog F56).—The portal tract, toward the top left of the picture shows no abnormality. The thickening of the reticular fibers around the fatty centrolobular cells, together with the infiltration of fibrocytes and round cells in the latter region typifies early fibrosis. Hematoxylin and eosin; × 140.



Figures 4, 5 and 6

(See legend on opposite page)

TABLE 2—*Hepatic Lesions of Alcohol-Treated Dogs*

Dog	Duration of Experiment, Weeks	Body Wt. Kg.		Liver		Microscopic Changes			
		Initial	Final	Weight, Gm.	Total Fatty Acids, Per Cent	Fat	Hepatic Fibrosis	Cirrhosis	Comment
F31	9	8.9	7.6	437	11.5	1+; globules*	0	0	0
F32	130	8.5	4.6	335		4+; globules*	4+†	+	Simultaneous periportal and perihepatic vein fibrosis†
F51	10	7.6	8.1	437	6.6	1+; scattered globules	1+	0	Bilelike pigment in liver cells around central vein and sublobular divisions of hepatic veins
F52	72	9.3	11.4			4-5+*	4+	3+	Simultaneous periportal and perihepatic vein fibrosis†
F53	43	9.4	10.5	814	24.2	3-4+*	2+	0	Congestion and small hemorrhages around central fatty zone
F54	158	12.0	13.3	542	14.7	4-5+; droplets and globules*; marked variation in different lobes	0	0	Marked congestion and edema with occasional hemorrhage around sublobular veins
F55	53	8.5	9.5	700		5+*	1+	0	Abundant scattered giant cells
F56	30	10.2	13.0			3-4+*	2+	0	Extensive sinusoidal distention
F57	10	9.6	9.1	545	10.2	2+; droplets and globules	2+	0	Centrolobular hemorrhage and necrosis
F58	130	11.2	13.0	582	12.4	Trace—1+; droplets	0	0	0
F60	90	12.5	15.2	575	15.6	2-3+; droplets and globules	0	0	Scattered round cell foci with giant cells
F61	59	12.3	16.2	760	23.3	2+; mainly droplets	0	0	Centrolobular hemorrhages

*The fat globules were around the central vein or seen around sublobular divisions of hepatic veins.

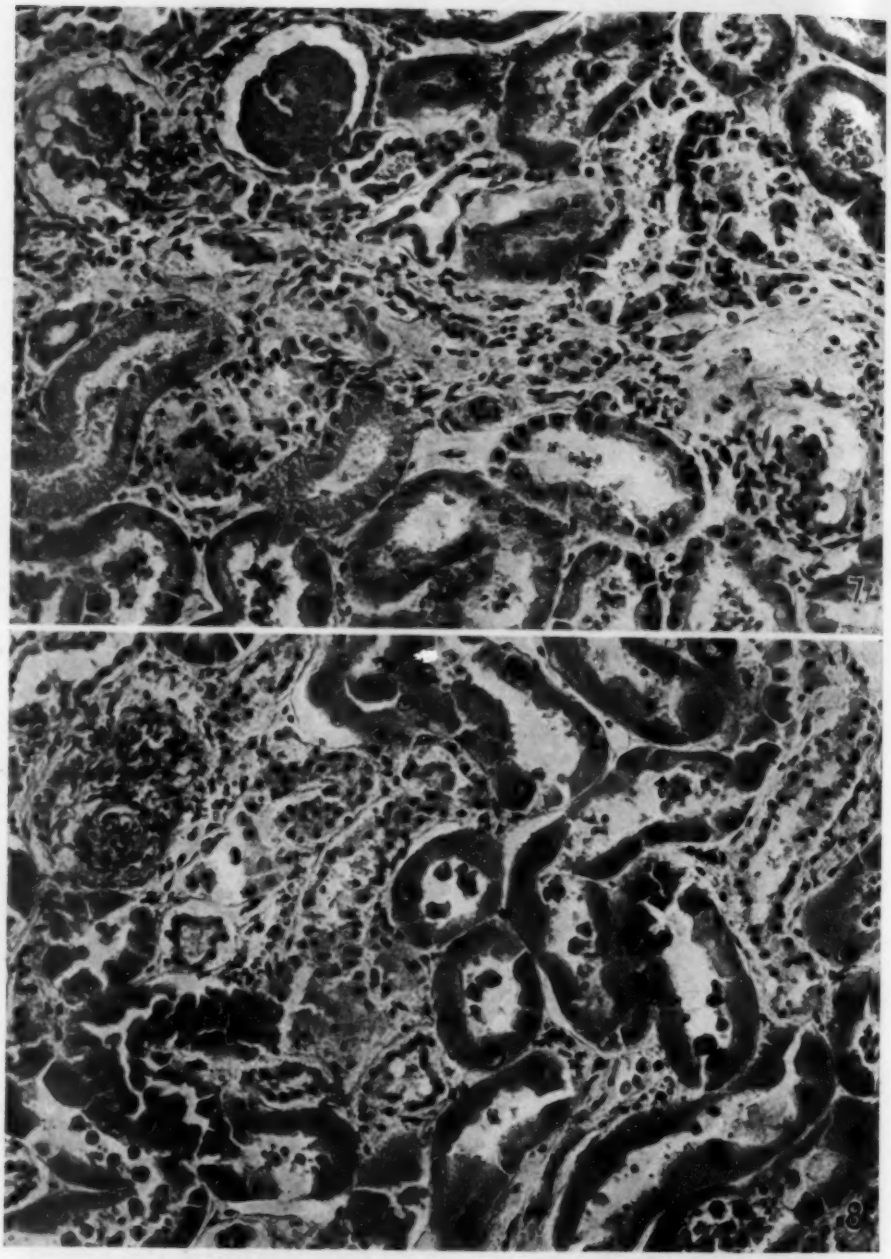
†The fibrosis developed around both the portal vein and the central vein or sublobular divisions of hepatic veins.

EXPLANATION OF FIGURES 4, 5 AND 6.

FIG. 4 (dog F56).—Edema, infiltration and slightly more advanced fibrosis are seen around this central vein as compared with figure 3. Note that the portal tract, on the left, is free from any fatty change, infiltration or fibrosis. The reaction in the centrolobular area is regarded as early fibrosis. Hematoxylin and eosin; × 80.

FIG. 5 (dog F52).—In this specimen the early centrolobular fibrosis has become more advanced and has extended farther into the lobule. The fibrous tissue is now more dense and the linking of the neighboring pericentral fibrotic areas has resulted in structural distortion, i.e., cirrhosis. Note the island of relatively fat-free periportal liver cells toward the bottom left corner of the picture with a normal, small portal tract. Hematoxylin and eosin; × 84.

FIG. 6 (dog F52).—Another portion of the liver of the same dog to show the severe fatty change and cirrhosis. The dense band of fibrous tissue here represents the scar tissue which has replaced a lobule. The fibrosis is also extending into the adjacent fatty liver tissue. Hematoxylin and eosin; × 65.



Figures 7 and 8

(*See legend on opposite page*)

Not only was the fibrosis observed around the hepatic radicles, but in dog F52, studied at seventy-two weeks, as well as dog F32, observed at the end of one hundred and thirty weeks, the portal tracts were also distinctly fibrosed. Nor was this portal fibrosis attributable to the passive incorporation of the portal tracts in the fibrosing hepatic radicles as in the reactions described in dogs deprived of both thyroid and pituitary glands.[10] In the alcohol-treated animals it seemed that the portal fibrosis commenced independently, for even the smaller tracts, which were well removed from fibrosed hepatic radicles, evidenced a distinct increase in young, cellular connective tissue. We are inclined to regard these livers as undergoing both periprotal and perihepatic vein cirrhosis simultaneously. However, it appears that the primary fibrosis occurs around the hepatic veins, for the extent and the degree of progress of the fibrosis were greater around the hepatic veins in these dogs. Furthermore, this same lesion was the only one present in those livers of alcohol-treated dogs which were not frankly cirrhotic.

A third group of 4 animals was studied at ninty (F60), one hundred and thirty (F32 and F58) and one hundred and fifty-eight (F54) weeks after the commencement of the administration of alcohol. The livers of 2 dogs, examined at ninety (F60) and one hundred and thirty (F58) weeks were only mildly fatty (table 2), the fat accumulating mainly in the liver cells of the inner halves of the lobules. In dog F54, which died after one hundred and fifty-eight weeks, the centrolobular fatty change was more marked but still unassociated with any significant alteration apart from some edema and congestion around the hepatic radicles. However, the liver of one of the dogs (F32), examined at one hundred and thirty weeks, displayed well marked precirrhotic changes of a nature identical with that of the changes described for dog F52; in addition, distinct cirrhosis was detectable in both the perihepatic and the periportal vein regions of the liver of this animal (F32).

Thus, fatty livers were present in all the animals of this series, being well marked in 6 of the 12 dogs; early fibrosis was detected in 7, while the livers of 2 animals were distinctly cirrhotic (table 2). It appears from this study that early fibrosis could be detected as soon as ten weeks after the commencement of the administration of alcohol, while definite cirrhosis could be induced within seventy-two weeks. On the other hand, since the livers of 3 dogs showed only fatty change even after receiving alcohol for as long as one hundred and thiry and one hundred and fifty-eight weeks, it is clear that there exists a distinct variation in the susceptibility of the dog's liver to the effects of prolonged ingestion of alcohol.

SOME PATHOLOGIC CHANGES ENCOUNTERED IN THE KIDNEYS OF DOGS RECEIVING ALCOHOL

In both of the animals in which hepatic cirrhosis was found (F32 and F52)

10. Chaikoff, I. L.; Gillman, T.; Entenman, C.; Rinehart, J. F., and Reichert, F. L.: To be published.

Explanation of Figures 7 and 8

Fig. 7 (dog F52).—Renal lesion in animal with hepatic cirrhosis, showing two fibrosing glomeruli toward diagonally opposite corners. A third glomerulus shows hyalinization with periglomerular fibrosis. Note the giant nuclei to the right of the darkly stained hyalinized glomerulus. Interstitial fibrosis is also clearly portrayed. Hematoxylin and eosin; × 235.

Fig. 8 (dog F32).—Here is shown the peculiar tubular change resuling in densely eosinophilic, nonvacuolated, enlarged convoluted epithelium of the tubules and the grossly enlarged cell nuclei. Note also in the tubule, toward the bottom right corner, that large and small cells and nuclei can occur in the same tubule. A sclerosing glomerulus is depicted toward the left edge of the picture, while tubules with microcells and nuclei can be seen near this glomerulus and toward the right edge. Hematoxylin and eosin; × 235.

and in an animal (F61) with only fatty change in its liver, severe and extensive renal changes were observed.

The glomeruli throughout the cortex were distinctly enlarged and extremely cellular. Frequently adhesions were observed between the glomerular tufts and the outer layer of Bowman's capsule (figs. 7 and 8). Usually with the appearance of such adhesions the glomeruli shrank in size and seemed to undergo rapid fibrosis. Stages of the formation of capsular adhesions and the schlerosis of the glomeruli are clearly shown in figures 7 and 8.

Occasional glomeruli showed thickening of the outer layer of Bowman's capsule with an associated periglomerular fibrosis. In such instances the entire glomerulus shrank and underwent hyalinization and fibrosis (fig. 7). Frequently an eosinophilic protein-like precipitate could be seen in the cavity of Bowman's capsule in both the swollen and the degenerating glomeruli.

In addition to these glomerular changes, patches of cortical hemorrhage with subsequent necrosis, round cell infiltration and fibrosis were not uncommon. Diffuse intertubular edema with early fibrosis was a frequent finding around the medullary collecting tubules (fig. 7).

Nor did the more highly specialized convoluted tubules escape, as is evident in both figures 7 and 8. Among the changes in the convoluted tubules, the most spectular was the presence of giant, densely eosinophilic, nonvacuolated epithelial cells bulging into the tubular lumen (fig. 8). In these enlarged cells the nuclei reached unusual proportions; the nuclear membrances were well defined, as were also the much enlarged nucleoli (fig. 8).

Together with such enlarged epithelial cells containing giant nuclei were found extremely tiny cells with almost pinpoint hyperchromatic nuclei (fig. 8). Entire segments of the convoluted tubules might be comprised of such dimunitive cells (see bottom left and bottom right corners of fig. 8). On the other hand, parts of some tubules were comprised of alternating groups of hypertrophied and shrunken cells (see tubule near bottom right hand corner of fig. 8). Interstitial fibrosis was usually distinct around such degenerating tubules. These changes in the convoluted tubules were most severe in the 2 dogs with well marked precirrhotic and cirrhotic lesions of the liver.

COMMENT

The interesting points emerging from this study are, first, the centrolobular fibrosis superimposed on fatty changes or hemorrhagic necrosis; second, the occurrence of both central and portal fibrosis; third, the erratic distribution of fibrosis in different lobes of the same liver, and, finally, the associated occurrence of renal lesions in these animals.

Until recently, centrolobular fibrosis was reported as occurring only in chronic hepatic congestion following long-standing cardiac failure in man[11] and in surgically induced hepatic congestion in the dog[12]. However, Lillie and associates[13] described centrolobular de-

11. (*a*) Lambert, R. A., and Allison, B. R.: Bull. Johns Hopkins Hosp. **27**:350, 1916. (*b*) Katzin, H. M.; Waller, J. V., and Blumgart, H. L.: Arch. Int. Med **64**:457, 1939. (*c*) Koletsky, S., and Barnebee, J. H.: Am. J. M. Sc. **207**:421, 1944. (*d*) Costero, I., and Borroso-Miguel, R.: Arch. Inst. cardiol. México **17**:337, 1947.

12. Zimmerman, H. M., and Hillsman, J. A.: Arch. Path. **9**:1154, 1930.

13. Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: Pub. Health Rep. **57**:502, 1942.

generation and fibrosis in dietary hepatic injury in rats. In their material the changes in reticular fibers preceding fibrosis were associated with the appearance of ceroid-laden phagocytes. Subsequently Ashburn and associates[14] conclusively demonstrated, by means of injection technics that the fibrosis induced in the livers of rats and guinea pigs by dietary or other means commenced in the centers of the hepatic lobules and not, as frequently maintained by other investigators, periportally. The reactions induced in the dog's liver by the experimental procedure outlined earlier in this paper were seen at so many different stages of development as to allow us to conclude that in these animals, too, hepatic fibrosis and cirrhosis commenced in the centers of the lobules. This observation substantiates the suggestion made by Ashburn and co-workers that the centrolobular fibrosis described by them was more common than hitherto recognized.

In several of our dogs the fibrosis started within a centrolobular area of hemorrhagic necrosis, which apparently developed acutely in a fatty liver as a result of some sudden alteration of the blood supply (fig. 2). In other animals, however, the centrolobular fibrosis followed some alteration of the reticulum in the fatty avascular centrolobular regions. Apart from the observations by Lillie and associates[13] and Ashburn and associates[14] quoted in the foregoing paragraph, such changes of the reticulum and the development of fibrous tissue that occurred in the central portions of the lobules have, to our knowledge, been observed only in chronic passive congestion of the liver[15] and following the vascular derangement induced by chronic histamine intoxication[16]. It seems possible that alterations of the blood supply of the central portions of the lobules resulting from congestion, chronic histamine intoxication and fatty change may play an important part in stimulating centrolobular fibrosis. Such circulatory factors may also be responsible in part for the erratic distribution of the fat, not only in different lobes of the same liver but even in different portions of a single lobe.

The fact that both periportal and centrolobular fibrosis can proceed simultaneously is another interesting point emerging from this and other studies[10]. In the experiments recorded here the centrolobular fibrosis was the commoner and apparently the earlier lesion. Circulatory disturbances and fatty change are apparently related to the centrolobular fibrosis, but we could not detect similar reactions to account for the portal fibrosis. However, our inability to detect in fixed tissues circulatory changes related to the portal tracts does

14. Ashburn, L. L.; Endicott, K. M.; Daft, F. S., and Lillie, R. D.: Am. J. Path. **23**:159, 1947.

15. Lambert and Allison.[11a] Katzin and others.[11b] Koletsky and Barnebee.[11c] Costero and Borroso—Miguel.[11d] Zimmerman and Hillsman.[12]

16. Eppinger, H.; Kaunitz, H., and Popper, H.: Die Seröse Entzündung, Berlin, Julius Springer, 1935.

not by any means eliminate their occurrence. This possibility can be excluded only by the application of more precise methods, such as those evolved by Knisely[17] and by Deysachs[18] for studying the hepatic circulation. Even the investigations of the hepatic circulation reported by Wakim and Mann[19] reveal the profound alterations of this circulation which appear within a short time after the application of a stimulus nocuous to the liver.

Similarly, alterations of the renal circulation, induced directly or indirectly by alcoholism or malnutrition, may account for the lesions detected in the kidneys of our dogs. This is suggested by the hemorrhagic necrosis induced in the kidneys of rats by dietary measures[20]. Such renal lesions may, however, be secondary to the hepatic damage. This possibility cannot be excluded in view of the frequency with which renal lesions are found associated with portal cirrhosis as reported by Baxter and Ashworth[21]. On the other hand, the report of Griffith and Wade[20a] and of Christensen[20b] of hemorrhagic necrosis occurring simultaneously in the liver and the kidneys in rats fed choline-deficient diets suggests that a nutritional etiologic factor may have been responsible for the pathologic changes found in both the livers and the kidneys of our dogs. Whether the disturbances of food intake induced by alcohol could alone have resulted in malnutrition in our dogs still remains to be determined.

SUMMARY

Severe hepatic and renal injury developed in dogs receiving alcohol while being maintained on a high protein diet. Whether the pathologic changes described were due to the alcoholization or to the associated malnutrition could not be determined.

The most common hepatic lesion was severe fatty change with or without centrolobular hemorrhage and fibrosis. Centrolobular hepatic fibrosis was superimposed on the fatty changes in the livers of 7 of the 12 dogs studied. In 2 dogs this fibrosis was severe enough to result in such gross structural distortion as to be regarded as frank cirrhosis.

Periportal fibrosis was associated with centrolobular fibrosis in 2 dogs. The genesis of these lesions is discussed, and the possible role of circulatory disturbances in their causation is stressed.

Glomerular damage and an unusual type of tubular damage were also detected in the kidneys of these dogs. Possible causes of these renal lesions are discussed.

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17. Knisely, M. N.: Anat. Rec. **71**:503, 1938; **73**:69, 1939.
18. Deysachs, L. J.: Am. J. Physiol. **132**:713, 1941.
19. Wakim, K. G., and Mann, F. C.: Anat. Rec. **82**:233, 1942.
20. (*a*) Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **131**:567, 1939. (*b*) Christensen, K.: Arch Path. **34**:633, 1942.
21. Baxter, J. H., and Ashworth, C. T.: Arch. Path. **41**:476, 1946.

PARAFOLLICULAR CELL ADENOMA OF THE THYROID GLAND

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HAMPERL,[1] in 1936, found a peculiar kind of cell in the salivary gland. The protoplasm was edematous, with fine granulation; the nucleus was small, shriveled, and placed mostly near the periphery. He saw these cells later in the external, secretory part of the pancreas, the parathyroid glands (oxyphil cells), the pituitary gland, the thyroid gland and the ovarian tubes. He called these edematous cells "oncocytes" and expressed the opinion that they originated as the result of a process connected with aging of the organism, in which they redifferentiated to a more primitive form. More or less similar cells were observed by others in the epithelium of the testicular tubules, in the prostate gland and the uterus, and in connective tissue.

Originating probably by transformation of characteristic cells of the single organs, these elements have not yet won their definite place in histologic classifications. There were attempts to place them within the endocrine system: Sunder-Plassmann[2] saw in them degenerate "neurohormonal" elements of the thyroid gland. Altmann[3] expressed the opinion that they are merely a variety of the parafollicular cells. On the basis of personal investigations[3a] I agreed with Hürthle,[4] Nonidez,[5] and Zechel[6] that the "oncocytes" of the human thyroid gland are an alternative form of the parafollicular cells.

Somewhat transformed, these cells may be found in tumors. According to Hamperl, the large cell adenoma of the thyroid gland (Langhans) consists of such cells. They have been found in: adenoma of the salivary glands, of the pancreas and of the kidney; adenolymphoma; bronchial carcinoid; suprarenal tumors. Zippel[7] described 5 cases of goiter of a type which histologically could be called "oncocy-

From the Neurosurgical Clinic, Serafimerlasarettet, Stockholm.

1. Hamperl, H.: Virchows Arch. f. path. Anat. **298**: 327, 1936.
2. Sunder-Plassmann, P.: Ergebn. d. Chir. **33**: 268, 1941.
3. Altmann, H. W.: Beitr. path. Anat. u. z. allg. Path. **104**:420, 1940.
3a. Bakay, L.: Virchows Arch. f. path. Anat. **314**:329, 1947.
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toma." In these cases the large cell adenoma formed small, sharply circumscribed nodules. According to the author, the cells forming the tumors were not identical with parafollicular or Langendorff colloid cells.

REPORT OF CASES

CASE 1. — A woman 38 years of age had noted a swelling of her neck since the age of 8 years and "heart pressure" in the last three months. She had lost 8 Kg. in weight within a year. She had been treated with iodine. Her developmental and nutritional state was medium. A goiter the size of a goose egg was palpated at the right, extending 1 fingerbreadth below the sternum. There were no ocular signs, no tremor. A systolic murmur was heard at the cardiac apex: The pulse rate was 80. The basal metabolic rate was + 16 per cent.

From the right side of the thyroid gland a mass was removed, which extended below the sternum, was the size of two male fists and in part was cystic. The left lobe was not increased.

Histologically, two kinds of tissue were found. There were follicles in limited quantity, containing a small amount of colloid. Large, evenly arranged giant cells were seen in the walls of the follicles. It was remarkable that the follicles were surrounded by numerous capillaries filled with blood and apparently adhering to the walls. The greater part of the nodule was of a compact structure. Large cells were found; their protoplasm was granular, and most of the nuclei were swollen and vesicular; in certain layers the nuclei were shriveled, richer in chromatin. Dilated capillaries filled with blood were seen, and in several areas hemorrhages. The cells were found in a perivascular arrangement and were around the capillaries as well as around the larger blood vessels. In this compact part no colloid was found.

CASE 2. — A woman 29 years of age complained of a swelling of the neck that had been present for three years, of violent cardiac palpitations, increasing dyspnea of one year's duration, with suffocations, of loss of weight and nervousness. Her developmental and nutritional state was poor. On the right side was a goitrous nodule the size of a hen's egg. There were no ocular signs. The skin was humid. There was light tremor of the fingers. Reflexes were increased. No cardiac deformation was noted. The pulse rate was 84. The basal metabolic rate was + 20 per cent.

A mass the size of a walnut was removed from the right side. The adenoma was surrounded by a capsule and sharply separated from the tissue of the thyroid gland. It showed a soft, somewhat papillary structure inside the capsule, yellower than usual.

Histologically, the greater part of the nodule was of a papillary structure, but with the papillae so pressed together that no interstitium was left (fig. 1). In some places the interiors of the papillae showed hyaline degeneration. The dilated lumen of a capillary formed the center of each papilla (fig. 2), and the cells were grouped around the capillary in the shape of a rosette. The section is similar to that of a follicle, but the colloid center is replaced by a dilated blood vessel. There was no colloid at all in this part. A few small follicles were embedded in the basal tissue. The epithelium of the papillae was composed of two or three layers and only rarely of one layer. The cells were large and swollen, and compressed one another into angular prismatic shapes. Their plasma was finely granulated, opaque, with clearer and darker shades. The nuclei were placed at the periphery, opposite the central capillary (fig. 3). While a majority of the nuclei were small, round, pyknotic, there were some which were larger, vesicular, irregular. Other parts of

the nodule showed the picture of a small-alveolar, large cell adenoma. The walls of the round microfollicles were lined with characteristic cells of a size far above the average, with edematous, vesicular nuclei and granular cytoplasm. These cells were of the parafollicular type, with frequent formation of giant nuclei and giant cells. The epithelium was always a single layer with a limited quantity of colloid. The

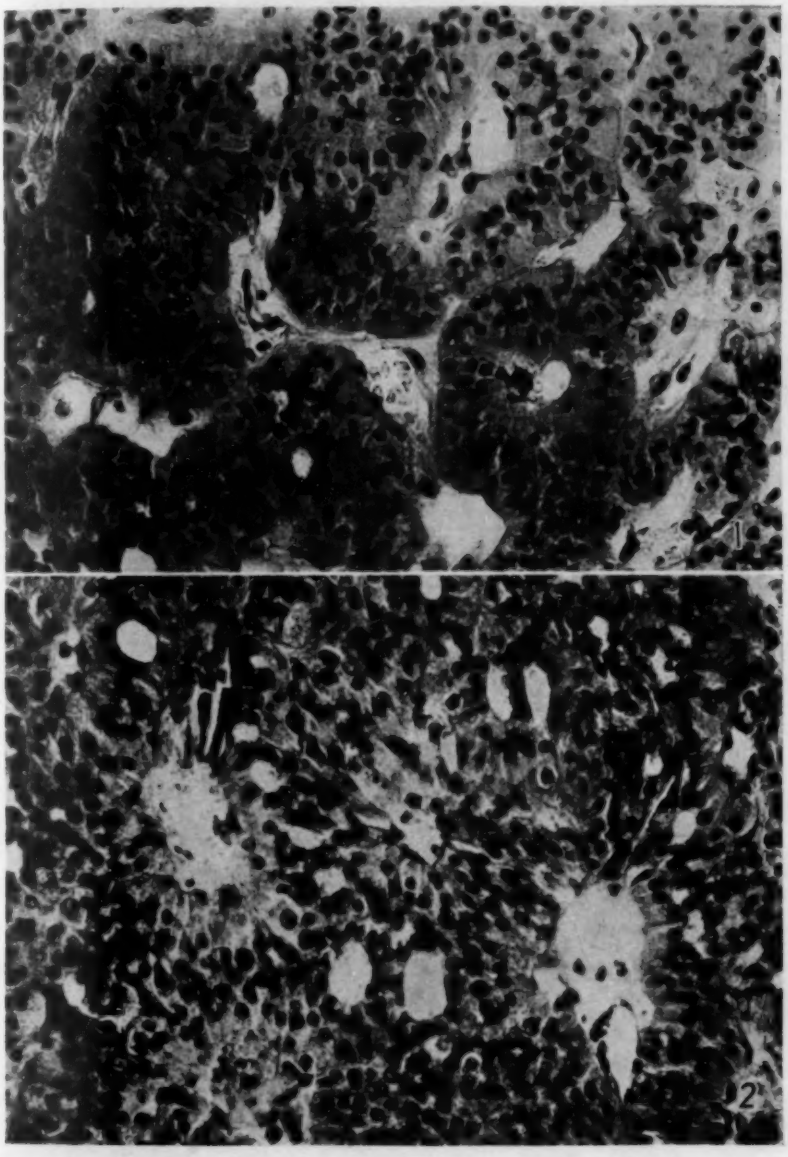

FIG. 1 (case 2).—Papillary structure of the thyroid gland; × 100. Note the dilated vessels among the papillae.

FIG. 2 (case 2).—Adenoma cells arranged around the dilated vessels; × 220.

disposition of the nuclei in these follicles was remarkable; they were placed not in the external or basal part of the cell but in the apical part near to the colloid (fig. 4).

CASE 3.—A woman 23 years of age had been ill three months with cardiac palpitations and dyspnea. Her dyspnea was independent of phyical work. There

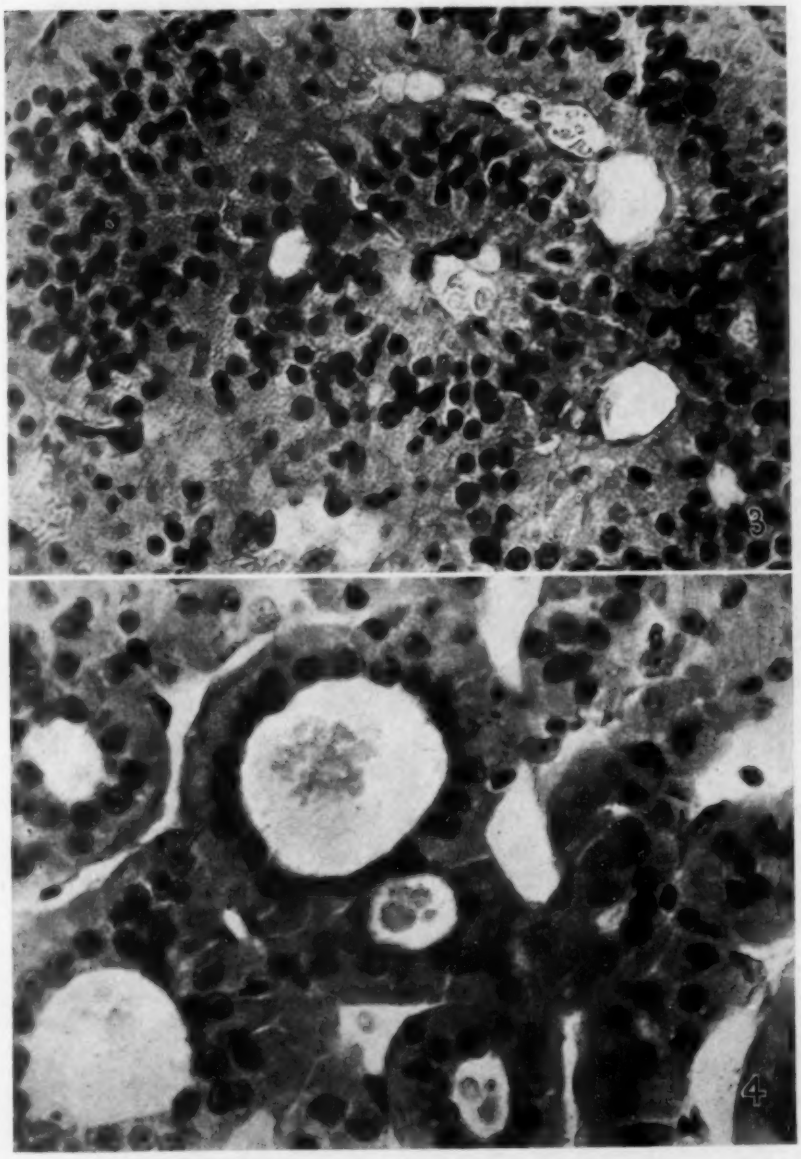

FIG. 3 (case 2).—Perivascular rosette; × 280.

FIG. 4 (case 2).—Follicular area of the adenoma; × 350. Each of the nuclei is in the apical part of its cell.

had been swelling of her neck for three years, with rapid increase in size during the last year. Her developmental and nutritional state was medium. The pulse rate was 78. The menses since the age of 18 had been regular. A nodule the size of

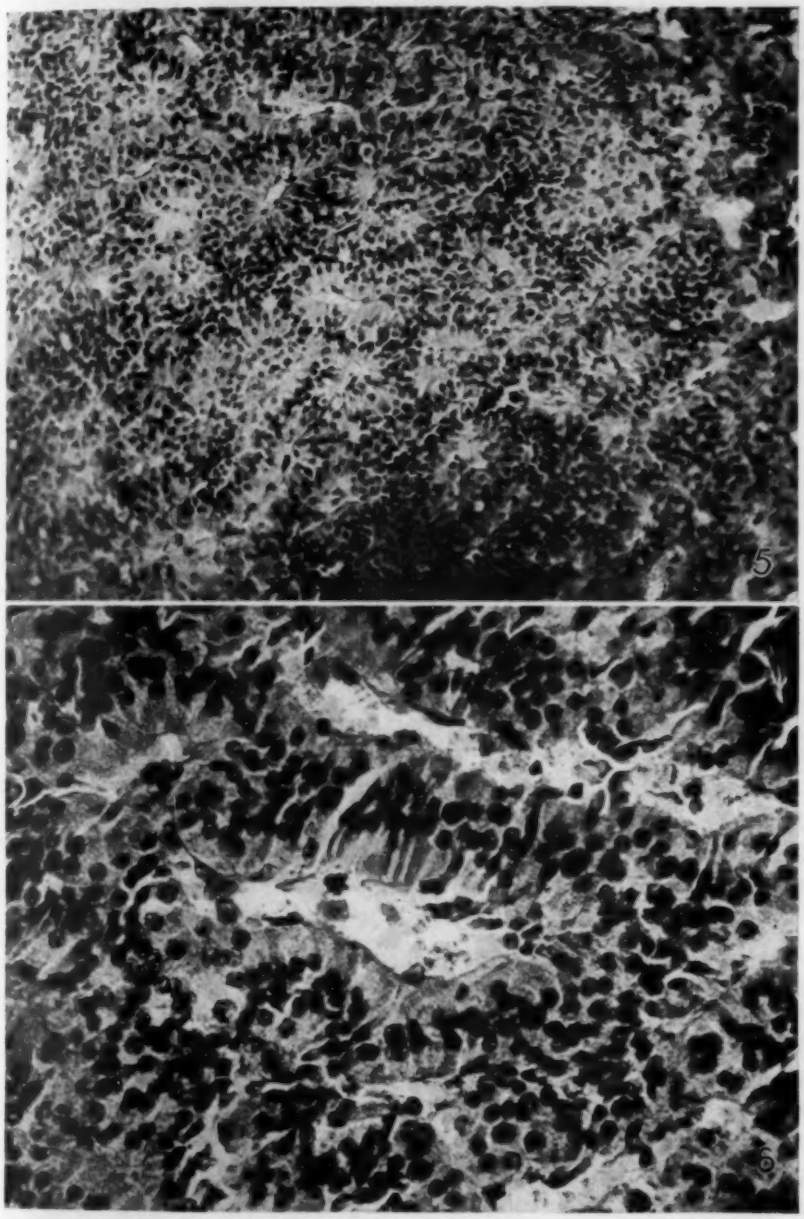

FIG. 5 (case 3).—Characteristic adenomatous structure; × 45.

FIG. 6 (case 3).—Columns of cells surrounding the blood vessels of the adenoma; × 250.

an egg was visible in the right lobe of the thyroid gland. The basal metabolic rate was + 8 per cent. Neurologic examination showed a neurotic reaction type developed on a psychogenic basis, which may have acted as a provocative, supporting factor for her thyrosis. No organic neurologic divergence was noted. A nodule the size of a small apple was removed from the right lobe of the thyroid gland. The left lobe was of normal size.

Histologically, one saw only a few follicles within the solid parenchymal tissue, and the nodule was almost entirely free of colloid (fig. 5). The nuclei of the majority of the cells were vesicular; the cytoplasm was edematous. The cells showed perivascular grouping and were similar to the types shown in figure 6.

Comparing the microscopic pictures in the 3 cases, one is able to follow, step by step, the formation of the adenoma. In the first phase of the transformation of the thyroid tissue the cells form small follicles containing only small quantities of colloid. Then the cells increase in size, as do their nuclei. They are, without doubt, regular cells of the thyroid gland, but in their new shape they remind one of parafollicular cells; however, they are without the increased activity of the latter, as indicated by the benign clinical symptoms. The dark variety of the parafollicular cells, which appears, according to my experience, in exophthalmic goiter and hyperthyroidism, is lacking among them. Even in this stage numerous blood vessels, mainly capillaries, appear in the thyroid gland; they surround the small follicles with their network. The polarity of the cells is directed against the capillaries, and the nuclei are pushed to the opposite side, near to the colloid. As the thyroid structure is gradually transformed into that of the adenoma proper, the cells increase in size and their nuclei shrivel. In this stage they correspond to the cells described by Hamperl. However, there is much variation in the structure of this tissue. The cells are grouped around the blood vessels. The follicles are dissolved; the colloid disappears entirely. The cells are arranged in the shape of a rosette around each of the capillaries. One is reminded of the mutual relation of the liver cells and the central vein. The angiopolarity becomes complete; the protoplasm of each cell is directed toward the lumen of the capillary and the pyknotic nucleus withdraws to the farther end.

The blood vessels play an important part in the adenoma, but the significance of the angiopolarity is so far not clear. It is probable that the cells evacuate their product directly into the blood stream. Clinical study showed slightly increased activity of the thyroid gland; the sensibility of the nervous system was increased. This much is certain. Angiopolarity is a process contrary to the regular secretion of colloid, for simultaneously with the perivascular arrangement of the cells, the acini and the colloid tend to disappear.

The large cell adenoma forms, in the tissue of the thyroid gland, sharply outlined nodules containing cells of abundant protoplasm

and large nuclei, which are fairly uniform and different from the cells of the thyroid gland. According to Getzowa,[8] they are of postbranchial origin, while Langhans[9] saw them in some cases of colloid goiter. The large cell goiter of Langhans, with small alveoli-forming metastases, belongs also to this type. Contrary to its name, in the majority of the cases the adenoma cannot be considered as cancer, and metastases are seen infrequently. The cells are large and eosinophilic, the protoplasma finely granulated, the nuclei sometimes shriveled. They form small round follicles, arranged sometimes within compact trabeculae of cells. The uniformity of the microscopic picture is remarkable. Wegelin,[10] too, considered these cells thyrogenic, the shrinking of the nuclei being obviously a symptom of degeneration. According to Altmann,[3] the Langhans cells of the struma are much like the parafollicular cells. Hamperl,[1] on the other hand, expressed the opinion that the cells of Langhans' struma are "oncocytes." However, the 3 cases of adenoma which I have described cannot be placed in such a classification. My observations induced me to consider the elements of this goiter as being related to the parafollicular cells on the basis of their structure and their cytologic peculiarities. The peculiar role played by the blood vessels in the structure of the tumors described gives these tumors a special position. In histologic appearance they are similar to the tumors termed by Wilensky and Kaufmann[11] as Hürthle cell tumors. The clinical data concerning 2 patients of the latter authors are similar to those reported by me; the metabolic rate did not increase. Reddish brown encapsulated nodules were removed by operation. There is a striking similarity between their figure 3 and my figure 6.

SUMMARY

Three cases of goiter of a new histologic type are described. The cells form rosettes around the capillaries, resulting in dense networks of a parenchymal structure and poor in colloid. The cells are large; their protoplasm is granular; their nuclei are vesicular, swelling at the beginning and later shrinking. They are similar to the "oncocytes" described by Hamperl, which I consider to be degenerate varieties of the parafollicular cells of the thyroid gland. The type of goiter described here is identical with the Hürthle cell tumor of Wilensky and Kaufmann but different from the Langhans adenoma.

8. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907.

9. Langhans, T.: Virchows Arch. f. path. Anat. **189**:69, 1907.

10. Wegelin, C.: Schilddrüse, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, pp. 191 and 252.

11. Wilensky, A. O., and Kaufmann, P. A.: Surg., Gynec. & Obst. **66**:1, 1938.

CHLOROQUINE (SN-7618)

Pathologic Changes Observed in Rats Which for Two Years Had Been Fed Various Proportions

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AND

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CHLOROQUINE (SN-7618) [1] is one of the few highly promising antimalarial drugs found among the thousands tested during the last few years. Chemically it is 7-chloro-4-(4-diethylamino-1-methylbutylamino) quinoline. In the available literature we have found only a few short statements concerning pathologic changes caused by chloroquine in animals studied as long as twenty-one weeks.[2] Because of the probability that there may be long-continued use of the compound in some cases, this two year study was undertaken.

The present paper deals with the gross and microscopic pathologic changes in 100 albino rats fed the free base chloroquine at various levels in their diet for two years. The nutritional aspects of this study and the effects on growth, mortality and the blood picture will be given in a separate paper.[3]

MATERIAL AND METHODS

Five groups of Osborne-Mendel albino rats, each group composed of 20, evenly divided as to sexes, were fed chloroquine mixed with their synthetic diet, at levels of 1,000, 800, 400, 200 and 100 parts per million (p.p.m.), beginning at 3 weeks of age and continuing for two years or until the animal died or was killed because of poor condition. A sixth group of 20 rats fed the same died concurrently but without chloroquine served as controls. The approximate mean survival times in descending order of dosage were 15, 32, 70, 80, 85 and 85 weeks respectively. Of the 120 animals, including the controls, 101 were examined microscopically. Tissues routinely sectioned included lung, heart, liver, spleen, pancreas, stomach, small in-

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency.

1. Loeb, R. F., and others: J.A.M.A. **130**:1069, 1946.

2. Wiselogle, F. Y.: A Survey of Antimalarial Drugs, 1941-1945, Ann Arbor, J. W. Edwards, 1946, vol. 1, p. 389.

3. Fitzhugh, O. G.; Nelson, A. A., and Holland, O. L.: To be published.

testine, colon, kidney, adrenal gland, testis (or uterus and ovary) and thyroid gland. Parathyroid glands, voluntary muscles, leg bones and bone marrows of about half of the animals were sectioned. A number of lymph nodes were incidentally included with other tissues. The standard treatment of the tissues included fixing in formaldehyde solution U.S.P., paraffin embedding and hematoxylin-eosin staining. The tissues of a few animals were fixed in Zenker's fluid, and a few sections were differentially stained to show collagenous fibers. Frozen and paraffin sections were stained for fat and ceroid respectively with sudan IV or oil red O. Perles' test for ferric iron, and carbol-fuchsin staining for ceroid, were done on paraffin sections. Unstained frozen sections mounted in water were examined between crossed Nicol prisms for doubly refractile material.

GROSS PATHOLOGIC OBSERVATIONS

A rough division could be made between the groups of animals receiving 1,000 and 800 p.p.m. of chloroquine, on one hand, and the remaining groups, on the other, in regard to the incidence and severity of the gross abnormalities present at autopsy; the gross changes were marked and slight, respectively. Pronounced humping of the back was present in 4 and 6, respectively, of the 20 rats fed 1,000 and the 20 rats fed 800 p.p.m. of chloroquine. There was distinct pallor of the viscera in 12 of the 20 rats on 1,000 p.p.m.; some of the remainder had lesser degrees of the same change. At 800 and 400 p.p.m., only these lesser degrees were seen, and pallor was not noted at lower dosages except in 2 rats at 200 p.p.m. Along with the visceral pallor went a tan tinging, most pronounced in the pancreas, next most pronounced in the liver, and affecting the viscera generally.

Whitish cardiac mural thrombi ,always in the left atrium and ranging in size from 2 to about 7 mm. in diameter, were present in 7 rats of the 800 p.p.m. group and in no others. Otherwise, the heart did not give much indication grossly of the marked microscopic changes to be seen in it later. Hypertrophy and/or dilation was rarely seen and was not related to the thrombi. Five rats had considerable amounts of clear, pale yellow fluid in their pleural cavities; 3 of these were on 800 p.p.m. dosage, and 2 of these 3 had cardiac thrombi. However, no clearcut reason for the appearance of the hydrothorax was determined. All 5 rats were found dead, and the phenomenon may have been terminal. On the other hand, hydrothorax is not seen this frequently in similar rats in most of our other experiments.

The liver, apart from the color changes mentioned, was affected grossly to a rather slight degree. Minimal to moderate, usually slight, roughness of the surface (orange skin surface) was the most frequent hepatic lesion, and was present in one fourth or slightly more of the animals at the two highest dosage levels, and less frequently at the lower levels. Some of the rough livers had a slightly increased consistency.

The kidney surface frequently showed slight or moderate fine pitting. Even though such pitting was as frequent when the diet contained no chloroquine (control group) as at 800 p.p.m., although not quite as pronounced, the incidence at 800 p.p.m. is considered significant because of its much earlier appearance. The pitted kidneys showed no fibrosis on section.

All males in the two high dosage groups had markedly atrophic testes. At 400 and at 200 p.p.m. a majority of the males were thus affected, while at the lowest level the testes were about as in the controls. Slightly to markedly brown uteri, generally considered as evidence of vitamin E deficiency, were seen in a minority of the animals; they were most frequent at 800 p.p.m. Hair balls were seen in the stomachs of a minority of both treated and control animals.

Structures unaffected grossly by the feeding of chloroquine were lungs, spleen, adrenal glands, urinary bladder, ovary, thyroid gland, parathyroid glands, small intestine, colon, lymph nodes, blood vessels, skin, and bones of the extremities.

MICROSCOPIC OBSERVATIONS

As with the gross abnormalities, a rough division could be made between the groups of animals receiving 1,000 and 800 p.p.m. of chloroquine, on one hand, and the remaining groups, on the other, in regard to the incidence and the severity of the microscopic changes (table).

Damage to cardiac muscle fibers was marked at dosage levels of 1,000 and 800 p.p.m., slight at levels lower than these, and was perhaps outstanding among the individual lesions. In the ventricles a necrosis of slow tempo, with moderately vascular fibrous tissue replacing the muscle fibers (fig. 1), was the most prominent change. Uniformly at the two highest levels, from one fourth to one half of the ventricular mass consisted of this fibrous tissue. Collagenous fibers and fibroblast-like cells were the most prominent components of the fibrotic areas. Fragments of muscle fibers were present in moderate number. At the peripheries of the patches of fibrous tissue one or a few bits of muscle showing coagulative necrosis and/or ingestion by machophages were often visible. There were small numbers of rounded macrophages and very small numbers of polymorphonuclear leukocytes and mast cells. Some of the fibroblast-like cells should perhaps be considered as macrophages in view of the fact that they contained a small amount of finely granular grayish or light brown material in the paraffin sections and considerable fat in the frozen sections. Anitschkow "myocytes" were increased over their usual number only slightly if at all. In the atriums, fibrous tissue replacement of muscle fibers was less pronounced than in the ventricles, while rarefaction and, to a lesser degree, swelling of the muscle fibers (fig. 2) were more pronounced. The rarefied portions of the fibers contained irregularly granular material of moderately oxyphilic staining reaction, and no distinct pigment. The nucleus was generally to one side of a rarefied area. The rarefaction was present also in the striated atrial musculature extending along the large pulmonary veins. The heart valves showed no abnormality.

The cardiac thrombi showed a moderate degree of organization. Fresher portions were sometimes seen attached over the older ones. The artrial musculature adjacent to the thrombi showed changes more nearly like those described for the ventricles than those present elsewhere in the atriums.

Damage to voluntary muscle, while pronounced, was of somewhat lesser severity than that to cardiac muscle. There was the same sharp break in severity below a dosage level of 800 p.p.m. Histologic characteristics were essentially the same as those described for cardiac muscle except that no rarefaction of the muscle fibers was present. In the instances in which the condition was more pronounced, around 25 per cent of the muscle was replaced by fibrous tissue. In general, the muscles of the leg showed more damage than did the muscles of the neck or such other muscles as were incidentally sectioned. A section of vertebral column and surrounding muscles from one of the animals with hunchback showed the same marked fibrous tissue replacement as was present in the muscles of the legs of this animal. It can be easily imagined that the imbalance of muscle dynamics caused by the damage of the muscles would be sufficient to allow hunchback to develop.

Damage of the liver was on the whole moderate in degree. At 1,000 p.p.m. 2 of 16 microscopically sectioned animals were graded as showing marked, 13 as showing moderate and 1 as showing slight to moderate damage of the liver. At 800 p.p.m. a few showed moderate damage and the remainder had changes of lesser,

usually slight, degree. At 400 p.p.m. and below, only rarely did the liver show as much as slight to moderate damage, and in the great majority of instances there

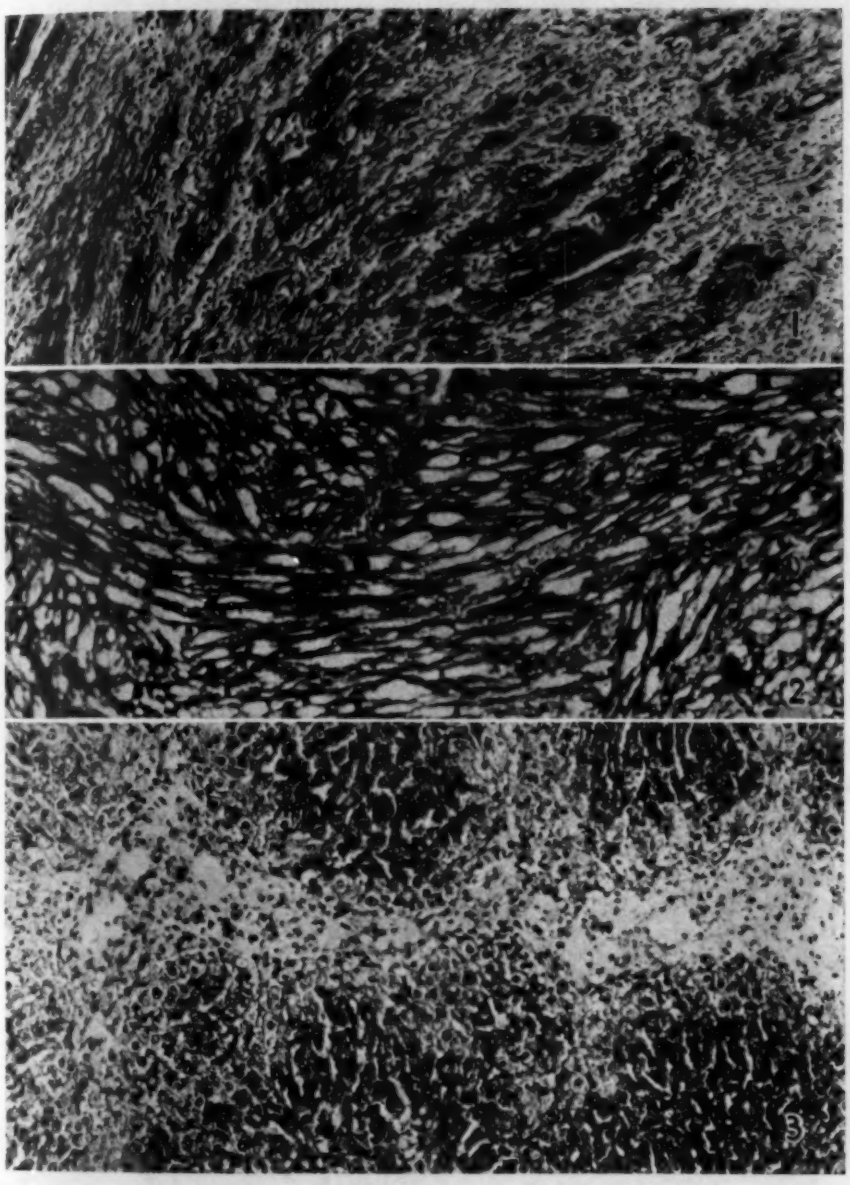

FIG. 1.—Ventricular myocardial necrosis and replacement fibrosis; × 125. Rat 6038; chloroquine in diet, 800 p.p.m.

FIG. 2.—Atrial myocardial rarefaction and slight swelling; × 125. Rat 6038, chloroquine in diet, 800 p.p.m.

FIG. 3.—Liver showing centrolobular necrosis with some fibrosis; × 125. Rat 6026; chloroquine in diet, 1,000 p.p.m.

was little or no variation from the controls. The hepatic lesions, other than the nonspecific ones of liver cell atrophy and bile duct proliferation, consisted most frequently of centrolobular necrosis or necrobiosis of hepatic cells and, consequent thereto, centrolobular fibrosis and distortion of structure. Sometimes the latter led

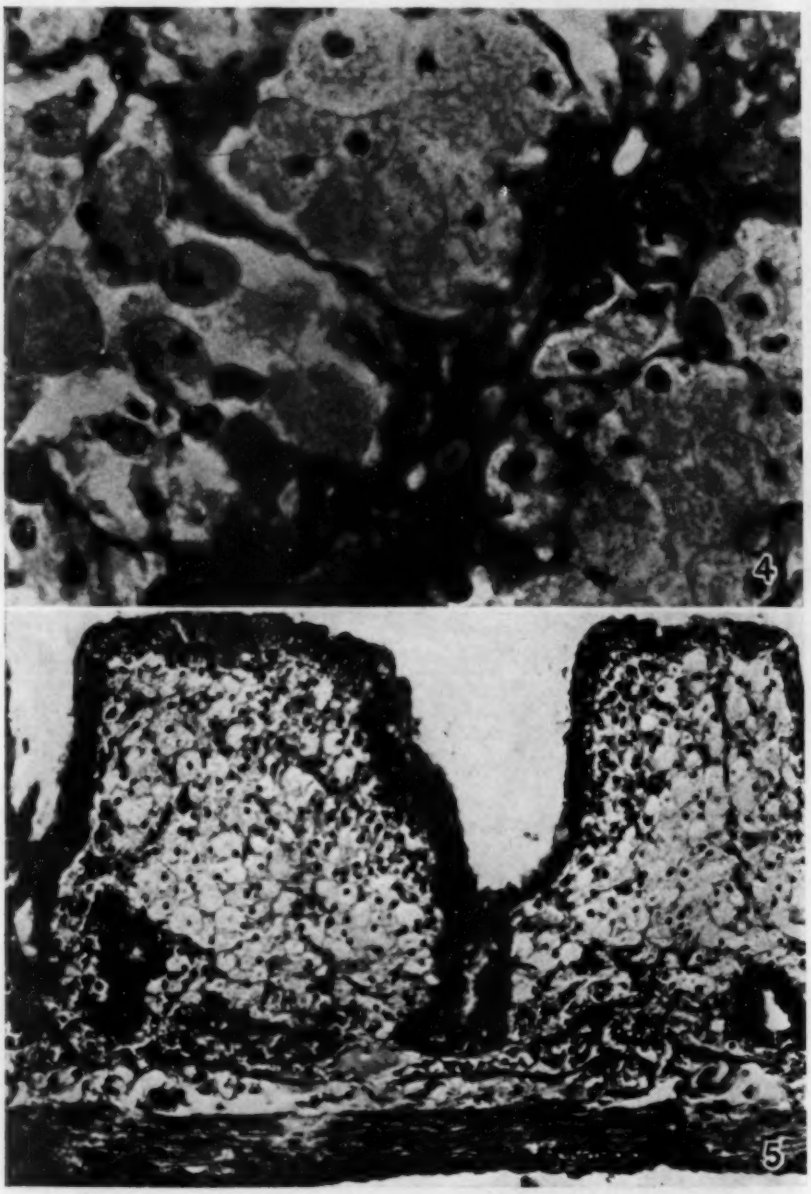

FIG. 4.—Focus of foamy macrophages filling pulmonary alveoli; × 708. Rat 6026; chloroquine in diet, 1,000 p.p.m.

FIG. 5.—Stroma of villi in small intestine filled with foamy macrophages; × 118. Rat 6048; chloroquine in diet, 800 p.p.m.

to a nodular appearance of the hepatic cell masses and merited the title of mild cirrhosis (fig. 3).

One of the nonlethal lesions, the most important as a group were the accumulations of foamy macrophages in various locations, including pulmonary alveoli, villi of the small intestine, and the splenic pulp. The pulmonary macrophages were distributed focally; those in the villi of the small intestine were diffused,

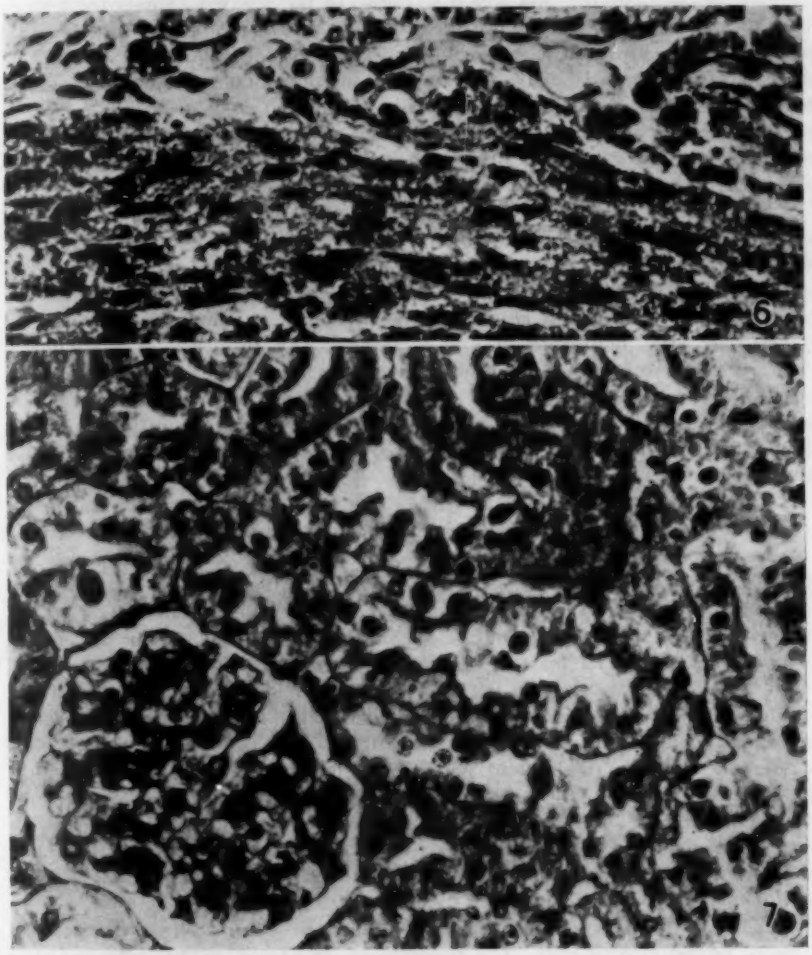

FIG. 6.—Pigment of ceroid type in myometrium and in adjacent macrophages; × 236. Rat 6047; chloroquine in diet, 800 p.p.m.

FIG. 7.—Nonferrous, nonfatty pigment globules in epithelium of renal convoluted tubules; × 472. Rat 6039; chloroquine in diet, 800 p.p.m.

but with their incidence increasing toward the cecum. In addition, parenchymal cells in one location, namely the renal tubules and of these chiefly the looped, took on a similar appearance. The macrophages in the lung and the small intestine were very similar (figs. 4 and 5) to each other. In the paraffin sections they measured from 10 to 25 microns and occasionally more in diameter, had abun-

dant, finely reticulated, foamy cytoplasm and had a relatively small, compact nucleus about the size of a red cell. In the paraffin sections the cytoplasm had no particulate content. In the lung the foamy macrophages appeared to arise from alveolar lining cells. Transitional stages were not prominent in the lung, but in the small intestine a transition into the ordinary type of macrophage was easily observed. The foamy macrophages in the spleen had more fragile cytoplasm than those elsewhere, so that they sometimes appeared as "holes," each containing a nucleus, in both paraffin and frozen sections. In the affected kidneys the cells of both the proximal and the distal looped tubules, and less frequently of the terminal portions of the proximal convoluted tubules and of the collecting tubules, were often greatly enlarged and rounded. Within the tubules were free rounded macrophage-like cells with similar cytoplasm and nuclei, often in castlike masses, in the same regions in which the attached foamy cells were located. All appearances suggested that the fixed cells desquamated or became dislodged into the lumens and then became rounded and sometimes larger.

Macrophages in all three locations and the enlarged renal tubular cells were seen in all or nearly all animals in the 1,000 and 800 p.p.m. groups, except that the pulmonary macrophages were present in but 6 of the 16 examined in the 1,000 p.p.m. group. At 400 and 200 p.p.m. the swollen renal tubular cells were still present in all or nearly all animals, although not so pronouncedly, while at 100 p.p.m. they were noticeable in only 1 animal. The incidence of macrophages dropped off more rapidly, in the small intestine sooner than in the lung and, especially, the spleen. In the spleen, the macrophages in animals receiving 200 and 100 p.p.m. (present in 8 and 3 respectively) did not appear until the ninetieth week of the experiment. The presence of these macrophages is not a normal old age phenomenon in our rats.

Atrophic renal tubules were present in both treated and control animals to account for the pitting of the renal surfaces noted grossly. Hyaline and calcified tubular casts were present proportionately especially at the lower dosage levels and in the controls, where the foamy cells did not block tubules.

The pigment observed, in addition to the usual moderate amount of splenic hemosiderin and the small amount of juxtamedullary pigment seen in the adrenal cortex, which is normal for our adult rats, was of two types. One type was of ceroid[4] nature and occurred in the uterus (fig. 6), and the other was nonferrous, and nonfatty, appeared yellowish brown in hematoxylin-eosin sections and occurred in the renal convoluted tubular epithelium (fig. 7). The two types of pigment were parallel in incidence (table) and were most prominent in the 800 p.p.m. group. The fact that but little pigment was seen in the 1,000 p.p.m. group, in which the rats survived on the average only half as long as those in the 800 p.p.m. group, indicates that some age or time factor was necessary for the deposition of these pigments. Both of these pigments were examined by several special staining technics; lack of space forbids presentation of the data obtained.

In the pancreas, cytoplasmic inclusions were seen within vacuoles in the basal portions of the acinous cells. The islands were uninvolved. The inclusions were neutrophilic to oxyphilic masses, up to about 15 microns in diameter, generally spherical, sometimes granular and sometimes homogeneous. In frozen sections they were moderately sudanophilic and showed no anisotropism. The inclusions were present in nearly all animals in the two higher dosage groups, and in 5, 3, and 3 respectively in the remainder.

The incidence of testicular atrophy was mentioned under the gross observations. There were no unusual histologic features. In the absence of paired-fed control

4. Endicott, K. M., and Lillie, R. D.: Am. J. Path. **20**:149, 1944.

animals we can make only an approximation of the role played by chloroquine as opposed to inanition in the production of the marked atrophy observed at the higher dosage levels. Our judgment is that both played a role. Direct toxicity of the drug was best seen in comparing the 200 and 100 p.p.m. groups, between which the factor of inanition did not come. Of the 10 males in each, 7 at 200 and only 2 at 100 p.p.m. had markedly atrophic testes, while 1 control was so affected.

Microscopic Pathologic Changes in Rats Fed Chloroquine

CHANGE Beyond Amount or Degree Present in Controls	Degree of Change at Given Dietary Level (Parts per Million) of Chloroquine				
	1,000	800	400	200	100
Myocardial damage	Marked	Marked	Slight	Almost none	Almost none
Damage of voluntary muscle	Moderate to marked	Moderate	Almost none	None	None
Damage of Liver	Moderate	Slight to moderate	Slight	Slight	Almost none
Testicular atrophy	Marked	Marked	Moderate to marked	Moderate to marked	Almost none
Foam cells in various locations	Many	Many	Moderate number	Small to moderate number	Few
Pigment in uterus	Little	Much	Little	Very little	Almost none
Pigment in renal tubules	Little	Much	Small to moderate amount	Very little	None
Inclusions in pancreas	Many	Many	Small to moderate number	Few	Few

Organs unaltered by chloroquine, except for changes of inanition, were thyroid gland, parathyroid glands, bone, bone marrow, stomach, colon, ovaries, adrenal glands and lymph nodes.

Examination for Fat.—This was done on 17 treated rats, including some from each dosage level, and 4 control rats. In the livers of the treated animals there was about the same amount of sudanophilic material as in the controls, and more doubly refractile material, regardless of dose level. In the kidney the cells of the convoluted tubules and the free and attached foamy cells in the looped tubules both showed small to moderate amounts of fat with the oil-soluble dyes, and little or no anisotropic material. Fat in the spleen varied from little to much more than the small amount in controls. In the heart there were small to moderate amounts of fat at 1,000 and 800 p.p.m. and little below this, correlating with muscle damage. The fat occurred in both muscle fibers and macrophages; anisotropic material was essentially absent. The intestinal macrophages showed a small amount of fat, tending to be less toward the lumen and more basally, with little anisotropic material. In one section containing pulmonary macrophages, both types of fatty material were absent. The fat content of the various pigments and inclusions has already been mentioned.

Examination for Glycogen.—A staining method designed to reveal glycogen in formaldehyde-fixed tissues was applied[5] to sections of lung, thyroid gland, liver and kidney from each of 2 rats showing typical lesions. The foamy macrophages and parenchymal cells had at the most a moderate glycogen content when compared with the hepatic cells. Otherwise, no unusual appearances were noted.

5. Through the courtesy of Dr. R. D. Lillie, National Institute of Health.

SUMMARY

Five groups of rats, each group containing 20, evenly divided as to sexes, were fed chloroquine (SN-7618), mixed with their diet, at levels of 1,000, 800, 400, 200 and 100 parts per million, beginning at 3 weeks of age and continuing for two years or until the animal died or was killed because of poor condition. A sixth group of 20 undosed rats served as controls. Gross examination of the tissues was done for all rats, and 101 of the 120 animals were examined microscopically in detail.

At the two highest dosage levels, the average life span was greatly shortened as compared with that at the lower dosage levels and that of the controls, and there were many gross and microscopic anatomic changes. The lesions observed grossly were humping of the back, pallor and tan tinging of the viscera, slight roughness of the liver surface, cardiac atrial thrombosis, hydrothorax, testicular atrophy, pitting of the kidney surfaces, and brown uteri.

Of the microscopic changes caused by chloroquine, the most damaging was a relatively slow necrosis of cardiac and voluntary muscle, with destroyed muscle being replaced by fibrous tissue. As much as half of the ventricular myocardium was so affected. In the atriums, rarefaction of the muscle fibers was more prominent than was fibrosis. Necrosis and replacement fibrosis of voluntary muscle, though still pronounced, was not as great as in cardiac muscle.

The liver was damaged to a moderate degree at the two higher dosage levels. Slow necrosis of hepatic cells led to centrolobular fibrosis and distortion of structure.

Macrophages with foamy cytoplasm were present in the lung, the small intestine and the spleen. Cells of the looped renal tubules hypertrophied into cells similar in appearance to the free macrophages. None of these cells had any distinct particulate content; they did contain some fat and glycogen. Pigment of ceroid type was present in the uterus, and a nonferrous, nonfatty pigment, in the renal convoluted tubules. Cytoplasmic inclusions were present in the pancreas.

In general, the 400 p.p.m. level was marked by a sharp drop in incidence and severity of lesions as compared with the two higher levels. At 100 p.p.m. changes from chloroquine were minimal.

Chloroquine caused a slight to moderate increase of fat in the liver, the kidneys, the heart, the spleen, the uterus and the pancreas, in the last two by virtue of the fat content of pigment and cytoplasmic inclusions respectively.

Organs unaltered by chloroquine, except for changes of inanition, were the thyroid and parathyroid glands, bones, bone marrow, the stomach, the colon, the ovaries, the adrenal glands and the lymph nodes.

DILATATION OF THE ACINI OF THE PANCREAS

Incidence In Various Pathologic States

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IN A RECENT histopathologic study of the pancreas[1], a remarkable degree of dilatation of the acini, flattening of the lining epithelial cells and inspissation of secretion occurring in various types of uremia was described. The lesion was observed in 39 per cent of 85 cases of uremia resulting from chronic glomerulonephritis, in 42 per cent of 85 cases of uremia resulting from hypertension (nephrosclerosis) and in 52 per cent of 100 cases of uremia resulting from miscellaneous causes. On combining all the cases of uremia without relation to cause it is apparent that the lesion was present in about 45 per cent. In a control series of 200 cases in which death did not result from uremia the lesion was observed in 20 per cent. The causes of death in the cases of the control series in which the lesion was presented are enumerated in table 1.

PROCEDURE

Because in these cases the histologic appearance of the pancreas was similar to that observed in so-called fibrocystic disease of the pancreas it was thought that further investigation of the lesion might be worth while. In order to obtain more information as to the incidence and genesis of the pancreatic lesion it was decided to investigate a large number of cases in which death resulted from the same causes as those listed in table 1. The methods used were essentially the same as those described in a previous study of the pancreas in uremia.[2] Because in the control series there were more cases in which death resulted from intestinal obstruction than from any other single cause, it was decided to examine sections of the pancreas in 50 cases each of carcinoma of the stomach, obstruction of the small intestines and obstruction of the large intestine. Sections of the pancreas also were examined in 50 cases each of cancer (exclusive of the pancreas and without gastric or intestinal obstruction), infections other than those involving the pancreas, congestive heart failure, intracranial lesions and chronic ulcerative colitis.

From the Section on Pathologic Anatomy, Mayo Clinic.

1. Baggenstoss, A. H.: Am. J. Path. **23**:908, 1947.

2. Baggenstoss, A. H.: The Pancreas in Uremia; A Histopathologic Study, Am. J. Path., to be published.

RESULTS

The incidence of the pancreatic lesion in each of these groups of cases is listed in table 2. The appearance of the lesion was identical with that described in a previous study.[2]

Carcinoma of the Stomach.—An example of the pancreatic lesion in a case in which death resulted from carcinoma of the stomach is shown in figure 1*a*. Dilatation of the acini of the pancreas occurred

TABLE 1.—*Pancreas in Uremic Dilatation of the Acini in 200 Controls*

CAUSE OF DEATH	DILATATION OF ACINI, CASES
Intestinal obstruction	11
Cancer	7
Infections	7
Congestive heart failure	6
Intracranial lesions	6
Chronic ulcerative colitis	3
Total	40

TABLE 2.—*Dilatation of Acini of Pancreas*

CAUSE OF DEATH	PRESENT				ABSENT			
	Cases		Mean Age Yr.	Vomiting, Per Cent	Cases		Mean Age Yr.	Vomiting, per Cent
	Number	Per Cent			Number	Per Cent		
Carcinoma, stomach	27	54	60	37	23	46	60	52
Obstruction, small intestine	21	42	46	67	29	58	53	62
Obstruction, large intestine	11	22	61	45	39	78	62	26
Infections	21	42	47	38	29	58	30	7
Chronic ulcerative colitis	21	42	34	29	29	58	52	41
Congestive heart failure	12	24	52	33	38	76	54	11
Cancer	11	22	61	27	39	78	54	8
Intracranial lesions	6	12	50	0	44	88	35	50

in 27 cases. It was of mild degree in 18 cases, of moderate degree in 7 and of severe degree in 2. There were 24 males and 3 females. The mean age was 60 years. Vomiting was among the symptoms in 10 cases.

In the group of 23 cases in which the lesion did not occur there were 18 males and 5 females. The mean age was 60 years. Vomiting was among the symptoms in 12 cases.

Obstruction of the Small Intestine.—An example of the pancreatic lesion in a case in which death resulted from obstruction of the small intestine is shown in figure 1*b*. Dilatation of the acini of the

pancreas occurred in 21 cases. It was of mild degree in 19 cases, of moderate degree in 1 case and of severe degree in 1 case. There

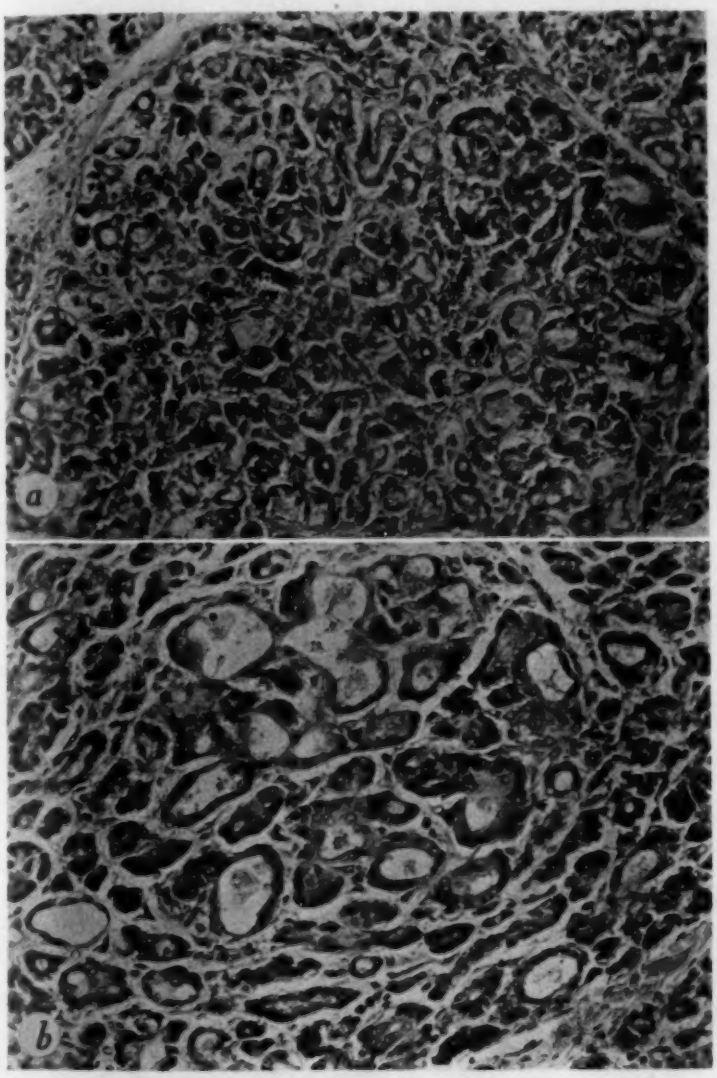

FIG. 1—Dilatation of the acini of the pancreas in (*a*) a case of carcinoma of the stomach (hematoxylin and eosin; × 110) and (*b*) a case of obstruction of the small intestine (hematoxylin and eosin; × 165).

were 11 males and 10 females. The mean age was 46 years. Vomiting was among the symptoms in 14 cases.

In the group of 29 cases in which the lesion did not occur there were 17 males and 12 females. The mean age was 53 years. Vomiting was among the symptoms in 18 cases.

Obstruction of the Large Intestine.—Dilatation of the acini of the pancreas occurred in 11 cases. It was of mild degree in 7 cases, of

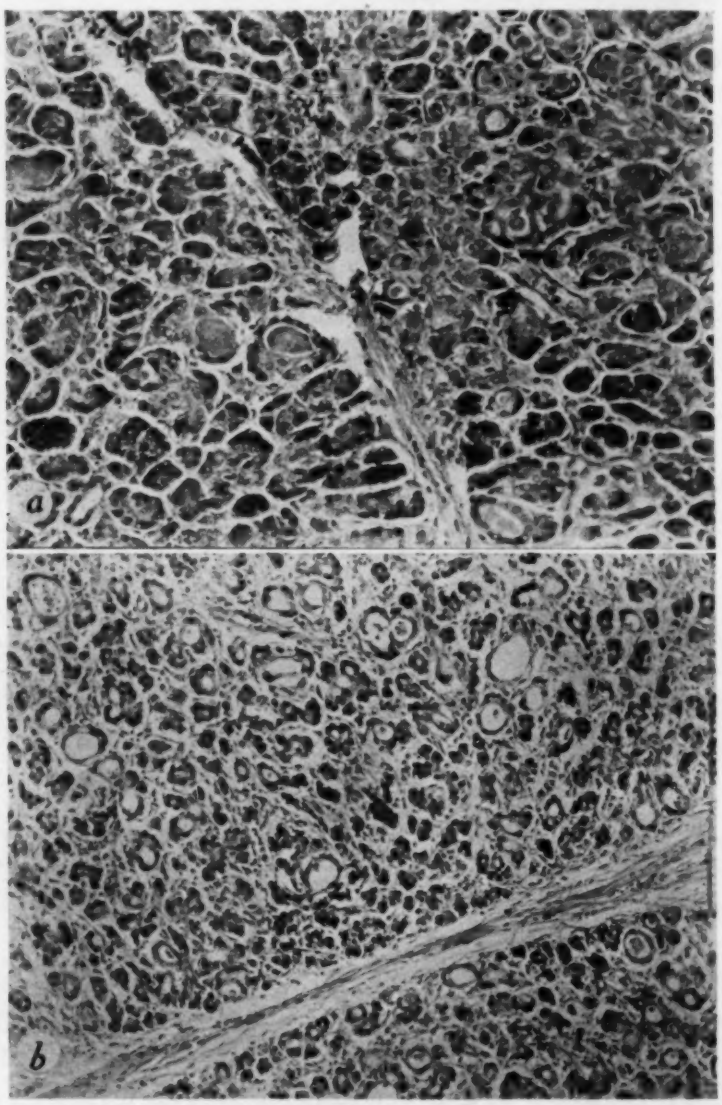

FIG. 2.—Dilatation of the acini of the pancreas in (*a*) a case of septicemia (hematoxylin and eosin; × 155) and (*b*) a case of chronic ulcerative colitis (hematoxylin and eosin; × 80).

moderate degree in 2 and of severe degree in 2. There were 6 males and 5 females. The mean age was 61 years. Vomiting was a symptom in 5 cases.

In the group of 39 cases in which the pancreatic lesion was absent there were 29 males and 10 females. The mean age was 62 years. Vomiting was a symptom in 10 cases.

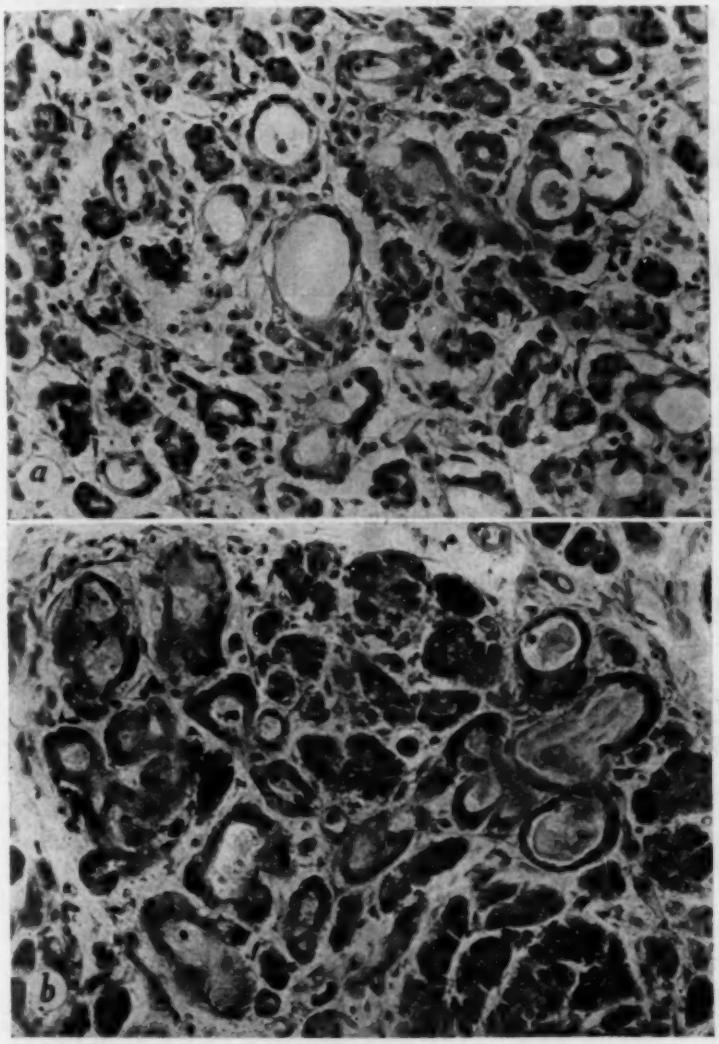

FIG. 3—Dilation of the acini of the pancreas in (*a*) a case of chronic ulcerative colitis (hematoxylin and eosin; × 200) and (*b*) a case of congestive heart failure (hematoxylin and eosin; × 200).

Infections (Sepsis or Septicemia).—An example of the pancreatic lesion in a case in which death resulted from septicemia is shown in figure 2*a*. Dilatation of the acini of the pancreas occurred in 21 cases. It was of mild degree in 13 cases, of moderate degree in 6 and of

severe degree in 2. There were 8 males and 13 females. The mean age was 47 years. Vomiting was a symptom in 8 cases.

In the group of 29 cases in which the lesion did not occur there were 22 males and 7 females. The mean age was 30 years. Vomiting was a symptom in 2 cases.

Chronic Ulcerative Colitis.—An example of the pancreatic lesion in a case in which death resulted from chronic ulcerative colitis is shown in figure 2*b* and 3*a*. Dilatation of the acini of the pancreas occurred in 21 cases. It was of mild degree in 16 cases, of moderate degree in 4 and of severe degree in 1. There were 7 males and 14 females. The mean age was 34 years. Vomiting was a symptom in 6 cases.

In the group of 29 cases in which the lesion did not occur there were 14 males and 15 females. The mean age was 52 years. Vomiting was a symptom in 12 cases.

Congestive Heart Failure.—An example of the pancreatic lesion in a case in which death resulted from congestive heart failure is shown in figure 3*b*. Dilatation of the acini of the pancreas occurred in 12 cases. It was of mild degree in 9 cases and of moderate degree in 3. In none of the cases was there a severe degree of dilatation of the acini. There were 6 males and 6 females. The mean age was 52 years. Vomiting was a symptom in 4 cases.

In the group of 38 cases in which the lesion did not occur there were 23 males and 15 females. The mean age was 54 years. Vomiting was a symptom in 4 cases.

Cancer.—In the group of 50 cases in which death resulted from cancer without obstruction of the gastrointestinal tract, dilatation of the acini of the pancreas occurred in only 11 cases. It was of mild degree in 10 cases and of moderate degree in 1 case. There were 5 males and 6 females, and the mean age was 61 years. Vomiting was a symptom in 3 cases.

In the group of 39 cases in which the pancreatic lesion did not occur there were 30 males and 9 females. The mean age was 54 years. Vomiting was a symptom in 3 cases.

Intracranial Lesions.—In the group of 50 cases in which death resulted from intracranial lesions, dilatation of the acini of the pancreas occurred in only 6 cases. It was of mild degree in 5 cases and of moderate degree in 1 case. There were 3 males and 3 females, and the mean age was 50 years. In none of these cases was vomiting listed as a symptom.

In the group of 44 cases in which the lesion did not occur there were 32 males and 12 females. The mean age was 35 years. Vomiting was a symptom in 22 cases.

COMMENT

These results clearly indicate that dilatation of pancreatic acini occurs with unusual frequency not only in cases of uremia but also in cases of carcinoma of the stomach, upper intestinal obstruction, sepsis and chronic ulcerative colitis. In cases of obstruction of the large intestine, congestive heart failure, cancer or increased intracranial pressure, the incidence of the pancreatic lesion is not significantly greater than in the original control series. The genesis of this pancreatic lesion is not known. In a previous report it was suggested that dehydration plus an interference with the release and normal action of secretin brought about by excessive vomiting was responsible for the lesion. Against the hypothesis that vomiting was the only cause is the fact disclosed in the present study that in the patients who died of carcinoma of the stomach, chronic ulcerative colitis or increased intracranial pressure the incidence of vomiting was lower in those with the pancreatic lesions than it was in the patients without the pancreatic lesions (table 2).

Physiologists have disclosed that inhibition of pancreatic secretion may be brought about not only by vomiting (Bernard[3], Bernstein[4]) but also by stimulation of the central stump of the vagus nerve (Pavlov[3]; Anrep[5]), stimulation of other sensory nerves (Gayet and Gillaumie[6]), rage and mental discomfort (Farrel and Ivy[7]) and distention of the stomach (Bizard and Boulet[8]). Distention of the stomach inhibits pancreatic secretion even during the administration of secretin. The inhibition occurs even when distention lasts only one or two minutes and, according to the experiments of the physiologists just mentioned, is the result of stimulation of the sympathetic nerves. Distention of the stomach is a common accompaniment of gastric carcinoma, obstruction of the small intestine and severe infections. Consequently, the fact that distention causes inhibition of the pancreatic secretion may be important in the genesis of these lesions. Unfortunately, these interesting observations regarding the inhibition of pancreatic secretion were not accompanied by histopathologic observations and consequently one can only speculate as to whether such inhibition might lead to the morphologic changes described in this report. It is interestng that in cases of obstruction of the large intestine the pancreatic lesion occurred in only 22 per

3. Cited by Lagerlöf, H. O.: Pancreatic Function and Pancreatic Disease, Stockholm, P. A. Norstedt & Söner, 1942.

4. Bernstein, cited by Gayet and Guillaumie,[6]

5. Anrep, G. V.: J. Physiol. **59**:1, 1914-1915.

6. Gayet, R., and Guillaumie, M.: Compt. rend. Soc. de biol. **112**:1058, 1933.

7. Farrell, J. I., and Ivy, A. C.: Am. J. Physiol. **78**:325, 1926.

8. Bizard, G., and Boulet, L.: Compt. rend. Soc. de biol. **116**:196, 1934.

cent whereas in cases of carcinoma of the stomach or of obstruction of the small intestine the figures were 54 per cent and 42 per cent respectively. This may be explained by the fact that distention of the stomach is less common in obstruction of the large intestine.

In a number of studies the morphologic effect, on the pancreas, of stimulation of the vagus nerves has been described. Babkin, Rubaschkin and Ssawitsch[9] noted that the acinous cells lost their granules and that the juice was rich in ferments and albumin after vagal stimulation. Sergeyeva[10] studied the pancreas of the cat after the vagus nerves had been subjected to the prolonged stimulation of an induction current and found that the acinous cells were almost entirely depleted of secretory granules and that the small ducts were distended with material stainable with the same dyes as the granules. A solution of hydrochloric acid introduced into the duodenum produced a flow of pancreatic juice, but histologic examination showed that the discharge of secretory granules of the acinous cells was slight.

Ramsay, Thomas and Crider[11] reported that if peptone is present in the intestine it stimulates the pancreas through a nervous mechanism which is dependent on the integrity of the vagus nerves. During the period of the stimulation the acinous cells become depleted of zymogen granules and secretion accumulates in the ducts.

In the photomicrographs accompanying the three studies just referred to, the histologic changes do not appear to resemble those which are the subject of this report. According to Farber[12], the lesion observed in so-called fibrocystic disease of the pancreas can be reproduced in kittens in all important respects by the injection of parasympathomimetic drugs.

Interesting observations have recently been reported from a number of sources, suggesting that malnutrition may be an important factor in producing the pancreatic changes described. Gilbert and Gillman[13] described the pathologic changes which occurred in rats fed on the deficient diet of corn pap and sour milk. In the pancreas the granules disappeared from the acinous cells, which lost their spheroid appearance and became arranged in the form of dilated ducts. These aggregations of ducts were scattered throughout the pancreas, and they could be seen with the naked eye as rather white opaque spots not larger than a pinhead. Gillman and Gillman[14]

9. Babkin, B. P.; Rubaschkin, W. J., and Ssawitsch, W. W.: Arch. f. mikr. Anat. **74**:68, 1909.

10. Sergeyeva, M. A.: Anat. Rec. **71**:319, 1938.

11. Ramsay, A. J.; Thomas, J. E., and Crider, J. O.: Anat. Rec. **86**:87, 1943.

12. Farber, S.: Am. J. Dis. Child. **64**:953, 1942.

13. Gilbert, C., and Gillman, J.: Science **99**:398, 1944.

14. Gillman, T., and Gillman, J.: Arch. Int. Med. **76**:63, 1945.

later stated that rats fed on mealy pap and sour milk commonly have cystic changes of the pancreas similar to those described and portrayed by Andersen in children. The rats also were found to have fatty livers, and this lesion preceded by many months the cystic changes that occurred in the pancreas. Friedman and Friedman[15] reported that in rats kept on high fat, low protein diets degenerative changes developed in the acinous tissue of the pancreas. These changes consisted of a loss of cytoplasm and a squaring of individual acinous cells. The cytoplasmic loss proceeded until only a thin rim remained about a vesicular nucleus. Separation of the acinous cells then occurred, leaving the parenchyma of the gland filled with separate cells having little organized relation to one another. In all cases the pancreatic changes occurred parallel with fatty infiltration and degeneration of the liver.

Mallory[16] found dilatation of the acini of the pancreas in approximately 10 per cent of the material that passed through his laboratory in Italy during World War II. He observed the lesion in patients who died from renal insufficiency and from severe infections, particularly typhus fever. Dehydration was considered an important etiologic factor.

In summary, it may be said that dilatation of pancreatic acini has been produced experimentally by parasympathomimetic drugs and by protein-deficient diets. It has been observed in cases in which death has resulted from uremia, severe infections, gastrointestinal obstruction, chronic ulcerative colitis or malnutrition. Malnutrition is common in carcinoma of the stomach, chronic intestinal obstruction and chronic ulcerative colitis. Malnutrition would not seem to be an important factor in many cases of uremia, acute intestinal obstruction or in severe infections; it may be postulated that in these cases the first or early stages of the lesion are the result of those conditions (gastric distention, vomiting, pain and emotional disturbances) that give rise to abnormal nervous stimuli which lead to inhibition of the type of pancreatic secretion normally aroused by secretin (a watery juice low in enzymes and proteins). Experimentally it has been shown that stimulation of either the vagus or the sympathetic nerves to the pancreas results in a discharge of the zymogen granules of the acinous cells and the production of a viscid juice with high nitrogen content. In such a set of conditions dehydration might result in inspissation of secretion and intrinsic obstruction of ductules and acini. In prolonged malnutrition a failure of reparative protein synthesis (cytoplasm and zymogen granules) may be the important factor in the genesis of the lesion.

15. Friedman, S. M., and Friedman, C. L.: Canad. M. A. J. **55**:15, 1946.
16. Mallory, T. B.: Am. J. Path. **23**:908, 1947.

The original purpose for which these studies were undertaken was to find if possible some clues to the etiologic factors of fibrocystic disease of the pancreas. The present study indicates that many factors are probably concerned in dilatation of pancreatic acini in adults, and the same may be true for the genesis of fibrocystic disease of the pancreas in children. As previously suggested, it may be that there is a congenital absence of secretin or some defect in the mechanism of its release. Another possibility is that secretin, though present and released, is destroyed before it can take effect. Greengard, Stein and Ivy[17] demonstrated that there is present in the circulation an enzyme which inactivates secretin. This substance, which they called secretinase, may be responsible for the refractoriness of some dogs to the action of secretin. If secretinase were present in abnormal amounts, might this also explain the pancreatic lesion observed in fibrocystic disease? An abnormality of this type might initiate the change, and chronic malnutrition, particularly protein deficiency, might be responsible for the progression and intensification of the pancreatic lesions.

If the hormonal (secretin) stimulation of the pancreas were inhibited (congenital absence; defect in mechanism of its release; excessive amounts of secretinase in the blood), then the only stimulation of secretion would be that due to nervous stimuli and that caused by pancreozymin. Such an unbalanced stimulation of the pancreas would result in a viscid juice which, if produced during a relative state of dehydration, might become inspissated and obstruct the ductules and acini. In fibrocystic disease of the pancreas the pancreatic juice is very thick and inspissated, and inspissation of bile and of the secretion of the intestinal glands also occurs (Farber[18]). Secretin stimulation of the pancreas has been described as causing a flow of alkaline fluid which serves to flush the alveoli, to thin the juice rich in organic material and to sweep it along the ducts (Best and Taylor[19]). There is some evidence also that secretin stimulates the secretion of bile and probably the succus entericus (Agren[20]). It has even been called an intestinal diuretic (Agren[20]). If secretin stimulation were absent in these cases, the obstruction of the small bile ducts and intestinal glands might also be explained as the result of the production and inspissation of an abnormally thick secretion. It would not explain the changes observed in the salivary glands (Farber[18]) or the lack of enzymes in the pancreatic juice (Ander-

17. Greengard, H.; Stein, I. F., Jr., and Ivy, A. C.: Am. J. Physiol. **133**:121, 1941.

18. Farber, S.: Arch. Path. **37**:238, 1944.

19. Best, C. H., and Taylor, N. B., in The Physiological Basis of Medical Practice, ed. 4, Baltimore, Williams & Wilkins Company, 1945, pp. 451-453.

20. Agren, G.: Skandinav. Arch. f. Physiol. **70**:10, 1934.

sen[21]; Farber, Schwarchman and Maddock[22]). Recent investigations by O'Neal[23] indicate, however, that small amounts of pancreatic enzymes may be present in specimens obtained by duodenal drainage in proved cases of fibrocystic disease of the pancreas.

SUMMARY

Dilatation of the acini of the pancreas occurs in an unusually large number of cases in which death results from carcinoma of the stomach, obstruction of the small intestine, severe infections or chronic ulcerative colitis. The genesis of the lesion is not known. It is suggested, however, that a number of factors may be of importance. Among these are (1) inhibition of the pancreatic secretion that is normally stimulated by secretin (the inhibition may be the result of gastric distention, vomiting or abnormal nervous stimulation); (2) nervous stimulation of the pancreas mediated through the vagus and sympathetic nerves, leading to depletion of zymogen granules and formation of a thick, viscid pancreatic juice; (3) dehydration, resulting in inspissation of the juice and the development of intrinsic obstruction, and (4) malnutrition (protein deficiency), resulting in progression and augmentation of the lesions due to a failure of the pancreatic cells to repair themselves. It is also suggested that a congenital lack of secretin, some defect in the mechanism of its release or excessive amounts of secretinase may be responsible for so-called fibrocystic disease of the pancreas.

21. Anderson, D. H.: Am. J. Dis. Child. **63**:643, 1942.

22. Farber, S.; Schwachman, H., and Maddock, C. L.: J. Clin. Inestigation **22**:827, 1943.

23. O'Neal, R.: Thesis, Graduate School of the University of Minnesota, 1948.

PNEUMONIA PRODUCED BY A MENINGOPNEUMOTROPIC VIRUS

Report of a Fatal Case, with Observations on the Interrelationship of Psittacosis-Like Viruses

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AND

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FROM THE LUNG of a patient with an unusual type of pneumonia which proved fatal, a virus was isolated in each of three attempts by inoculating material from the lung directly into mice and subsequently by inoculating a suspension of frozen human lung into chick embryos[1]. In the lesions produced by this virus elementary particles are present in abundance. The pulmonary lesions of the experimental disease are similar to those produced by viruses designated as causing meningopneumonitis,[2] ornithosis[3] and human pneumonitis,[4] as are the immunologic properties. A review of the anatomic changes of reported cases of human psittacosis, ornithosis and related diseases and a study of the experimental disease led to the conclusion that the essential alterations are the same in all and that their causative agents are closely related, if not identical.

THE HUMAN CASE OF FATAL PNEUMONIA

A 47 year old woman, white, Irish-born, single, employed as a domestic, had a febrile infection of the upper respiratory tract. Two weeks before she was admitted to the New York Hospital, she had a headache and a dry cough, occasionally productive of some blood-tinged sputum, and a week later shaking chills, but she continued to work until the day of admission. She had not been in contact with birds.

On admission her temperature was 40.3 C. (104.5 F.), the pulse rate 120, the respirations 40, and the blood pressure 180 systolic and 110 diastolic. She was

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1. The latter was done by Dr. Thomas P. Magill, who has given us permission to quote his findings.

2. Francis, T., Jr., and Magill, T. P.: J. Exper. Med. **68**:147, 1938.

3. (*a*) Meyer, K. F.; Eddie, B., and Yanamura, H. Y.; Proc. Soc. Exper. Biol. & Med. **49**:609, 1942 (*b*) Meyer, K. F.: Medicine **21**:175, 1942.

4. Eaton, M. D.; Beck, M. D., and Pearson, H. E.: J. Exper. Med. **73**:641, 1941.

obese and appeared acutely ill, breathing with short inspiratory gasps and with a prolonged expiratory wheeze. The pharynx was red and edematous. The lungs were resonant. There were crackling inspiratory rales over most of the chest, and expiratory rhonchi and wheezes over the left lung. Fluoroscopy revealed a diffuse haze throughout the right lung.

Laboratory Examinations.—The total white blood cell count varied between 6,500 and 8,000, with a moderate "shift to the left." The urine contained albumin (1 or 2 plus). Blood cultures made on the first and fourth hospital days were sterile. Cultures of material taken from the throat on admission yielded nonhemolytic Staphylococcus aureus, alpha and gamma streptococci, Hemophilus influenza and Micrococcus pharyngis siccus, but no pneumococci. No cold agglutinins were present in the blood serum on the first hospital day. Blood obtained post mortem agglutinated in a dilution of 1:20 (+) the indifferent Streptococcus no 344 of Thomas and associates.[5]

Course.—Sulfadiazine was given for four days, and blood levels of 7.2 to 9.4 mg. per hundred cubic centimeters were attained. The temperature remained between 39 and 40 C. (102.2 and 104 F.); dyspnea continued, and cyanosis appeared at the slightest exertion and was not relieved by oxygen treatment. Respirations became more shallow, and the patient became stuporous and died on the sixth hospital day.

Gross Anatomic Changes.—The woman was well developed and obese. There were approximately 50 cc. of yellow, turbid fluid in the peritoneal cavity and a few cubic centimeters of straw-colored fluid in the pleural cavities. The lungs weighed 2,000 Gm. All lobes contained gray red, ill defined or lobular areas of consolidation. A large amount of frothy fluid exuded from cut surfaces of nonconsolidated areas. Suppuration was absent. The bronchi contained a moderate amount of viscous brown material. The lower lobe of the left lung was red and almost entirely consolidated. Approximately one half of the upper lobe of the left lung, three fourths of the lower lobe of the right lung and about one half of the middle lobe of the right lung were consolidated. There were spotty areas of hemorrhage in both lungs. The tracheobronchial, and cervical lymph nodes were slightly enlarged and soft. The spleen weighed 470 Gm. and was soft.

Microscopic Observations.—The pulmonary changes were essentially the same in all lobes. The pneumonic consolidation was predominantly alveolar and varied in intensity in different parts of the lungs. In some places there was massive interstitial or alveolar edema, with only a few inflammatory cells, while in other places the consolidation was massive. It was characterized by the presence of large numbers of mononuclear cells and of fibrin, with a variable number of erythrocytes (figs. 1 to 3). The septums were merely congested, and contained an increased number of leukocytes within the capillaries or in the process of migrating into alveoli. Among the cells of the exudate, lymphocytes and monocytes were predominant. Large numbers of cells of the alveolar lining were desquamating (figs. 1 and 2). The number of these cells was greater than might be expected from desquamation alone, and it seemed probable that they had proliferated. In areas with abundant edema fluid, an eosinophilic hyaline membrane lined many alveoli. The mucosa of many bronchi was swollen. The epithelial cells were desquamated. The submucosa was edematous and contained many lymphoid cells and occasional erythrocytes. Small collections of lymphoid cells were seen about large vessels. A few bronchi contained plugs of fibrin and mononuclear and polymorphonuclear leukocytes. There was a moderate serum precipitate in the interlobar septums.

In imprints stained with Giemsa's stain, the predominant cells were lymphocytes, monocytes and epithelial cells and a few polymorphonuclear leukocytes. Most of

5. Thomas, L.; Mirick, G. S.; Curnen, E. C.; Ziegler, J. E., Jr., and Horsfall, F. L. Jr.: Science **98**:566, 1943.

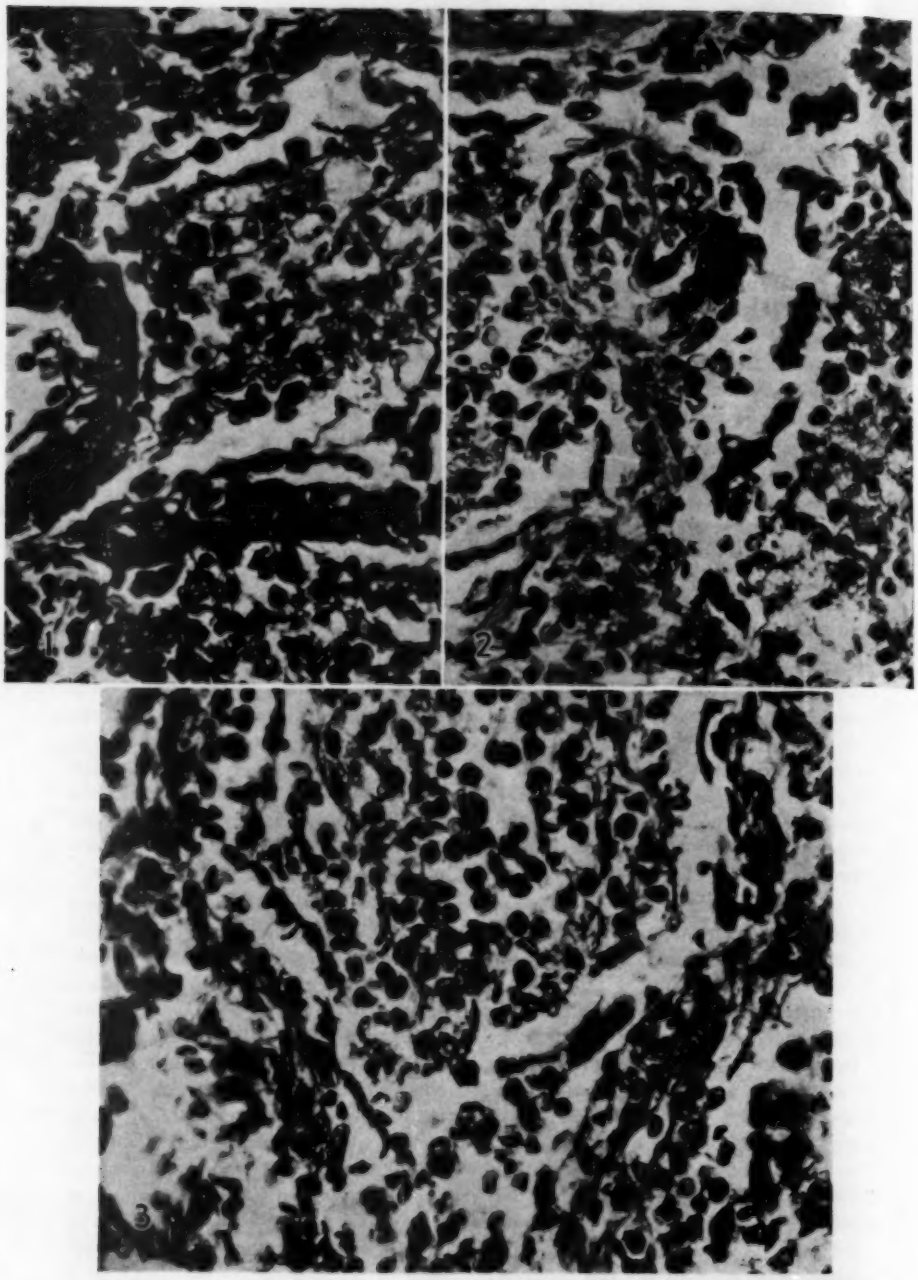


FIG. 1.—Section from a lung of the patient with an unusual type of pneumonia. The alveoli contain fibrin and mononuclear cells. The lining cells are in the process of desquamation. Hematoxylin-eosin; × 200.

FIG. 2.—Section from the same lung, showing detached alveolar lining cells, mononuclear exudate and erythrocytes in an alveolus and about a small vessel. Hematoxylin-eosin; × 200.

FIG. 3.—Section from the same lung. There are numerous mononuclear cells and erythrocytes and some fibrin in the exudate. Hematoxylin-eosin; × 200.

the large mononuclear cells were vacuolated and free from inclusion bodies. Several large monocytes contained innumerable minute pale-staining coccoid bodies. The size of these was smaller than that of the azurophilic granules of monocytes. In one smear a few diplococci were seen. The epithelium of the trachea was desquamated in most places, and only fringes of cuboidal epithelial cells lined the submucosa. The latter was infiltrated with lymphoid cells. There was slight disintegration of the epithelial cells of the mucosal glands.

A section from the abdominal muscle showed massive hemorrhage, with focal areas of necrosis of muscle fibers. The sinuses of cervical lymph nodes were distended with lymphocytes and monocytes, and moderate numbers of erythrocytes. No noteworthy changes were found in other organs examined.

Bacteriologic Examinations.—Examinations were made in the Central Laboratories of the New York Hospital: Cultures of heart blood and of the upper lobe of the left lung and the middle lobe of the right lung were sterile. Cultures of pus from the conjunctivas and of abdominal fluid yielded Staphylococcus aureus; and from the lower lobe of the right lung, Staph. aureus and alpha nonhemolytic streptococci.

Bacteriologic findings in Dr. W. G. Smillie's laboratory: Hemolytic Staph. aureus was found in cultures of material taken from the nasopharynx and the lower lobe of the right lung and of heart blood, while cultures of material from the middle lobe of the right lung remained sterile, and those of material from the upper lobe of the left lung yielded a diphtheroid organism. A green-producing streptococcus was isolated from the nasopharynx, and two varieties of a similar streptococcus were obtained from the trachea. None was isolated from the lungs, Neufeld's *Quellung* test made with Streptococcus no. 344 (5) immune serum showed no swelling of the capsules of these three strains of Streptococcus. The streptococcus isolated from the nasopharynx, however, gave positive microscopic agglutination with this serum; the other two strains did not.

ISOLATION OF VIRUS

Three successive attempts were made to isolate a virus pathogenic for mice from the patient's lung (table 1). Each of these yielded a meningopneumotropic virus with identical characteristics.

Technic.—The material and the viruses studied were preserved either in the frozen state in a carbon dioxide icebox at approximately —70 C. or were dried from the frozen state. The frozen material was thawed and ground in a mortar with crystalline aluminum oxide under aseptic precautions. A volume of sodium chloride solution equal to ten times the weight of the organ was added, and the resulting suspensions were centrifuged for five minutes at 1,000 revolutions per minute. The supernatant fluid was designated as a 10 per cent suspension.

Young mice, 3 to 5 weeks old, were used with few exceptions. After induction of light ether anesthesia intranasal instillations were made of volumes of 0.05 cc., and intracerebral injections of volumes of 0.03 cc. to 0.05 cc.

Experiment 1.—A 10 per cent suspension of the patient's lung was instilled intranasally into 3 Swiss mice. One of these, killed after seven days, had slight pneumonia, detected on microscopic examination only. Intranasal subpassages were made with lung suspensions at seven day intervals, as indicated in the table. Pneumonia was found in 2 mice after each of the first two passages, examined microscopically. In the next subpassage, pulmonary consolidation developed in all 3 inoculated mice, and elementary particles were identified in sections of the lungs of 2 of them. These particles are illustrated (fig. 7) and described in a subsequent

section. In the course of the subpassages, the virus gained in virulence, and in the fourth passage all 3 mice died of pneumonia five to six days after instillation of the suspensions (table).

Experiment 2.—In order to verify the results of experiment 1, a second inoculation experiment was made with suspensions prepared from lung that had been kept frozen at approximately −70 C. in sealed tubes in a carbon dioxide icebox for one hundred and ten days. In this experiment two different stocks of mice were used in addition to Swiss mice. The subpassages were made always within the stock. The virus was identified in fatal pneumonic consolidations in the second and subsequent passages.

Experiments in Which Virus M Was Isolated by Inoculating Mice with Lung of Patient M

Experiment	Passage	Route of Infection	Mice Inoculated		Gross Examination†	Microscopic Examination		
			Stock	No.		Mice Examined	Number with Pneumonia or Meningitis	Number with Elementary Particles
1	1*	Intranasal	Swiss	3	0	1	1	
	2	Intranasal	Swiss	3	0+2	1	1	
	3	Intranasal	Swiss	3	1+2‡	3	3	2
	4	Intranasal	Swiss	3	3	2	2	
2	1*	Intranasal	Swiss	3	0	1	0	
	1*	Intranasal	C_3H	3	0	1		
	1*	Intranasal	AK	4	0	1		
	2	Intranasal	Swiss	3	0	1	0	
	2	Intranasal	C_3H	3	0	1	0	
	2	Intranasal	AK	5	2+3	5	5	2
	3	Intranasal	Swiss	3	0	0		
	3	Intranasal	C_3H	3	0	0		
	3	Intranasal	AK	3	2+1	2	2	2
	4	Intranasal	Swiss	3	0	0		
	4	Intranasal	C_3H	3	0	0		
	4	Intranasal	AK	4	4	4		
	5	Intranasal	Swiss	3	0	0		
	5	Intranasal	C_3H	3	0	0		
	6	Intranasal	Swiss	3	0	0		
	6	Intranasal	C_3H	3	0	0		
3	1*	Intracerebral	Swiss	3	0+1‡	1	1	1
	1*	Intracerebral	C_3H	3	0	1		
	2	Intracerebral	Swiss	3	2+1	2		
	2	Intracerebral	Swiss	3	3	3	3	1
	2	Intracerebral	C_3H	3	0	0		
	2	Intracerebral	C_3H	3	0	0		
	2	Intracerebral	C_3H	3	0	0		
	3	Intracerebral	Swiss	4	3+1	1		
	4	Intracerebral	Swiss	3	2+1	0		

*This passage was made with a 10 per cent suspension of the patient's lung; the subsequent passages were made with suspensions of mouse lung in experiments 1 and 2 and with suspensions of mouse brain in experiment 3.

†The first figure indicates the number of mice that died from pneumonia following intranasal infection or from meningitis following intracerebral infection. The second figure indicates the number of killed animals that had pneumonia or meningitis.

‡Cultures of lung or brain were sterile.

Experiment 3.—Since tests of the virus isolated indicated that the minimal infective dose was smaller by the intracerebral route, a group of mice was given intracerebral injections of a suspension prepared from the patient's lung, frozen for one hundred and ten days. In Swiss mice the virus was isolated in the first passage.

Control Experiments.—The question whether the virus isolated is human or murine in origin necessitated numerous control experiments.

A. Serial intranasal subpassages were made with 10 to 20 per cent suspensions of normal mouse lungs in normal inbred mice of the stocks used in the foregoing study. Two experiments were performed with mice of stock AK, two with Swiss mice, and five with stock C_3H. The "blind" subpassages were made at 3 to 4 or at 7 day intervals, and from 4 to 17 successive subpassages were made. In no instance was a meningopneumotropic virus isolated.

B. Serial "blind" passages were made by the intranasal route in different stocks of mice, initiated (*a*) with throat washings from 2 normal persons, (*b*) with fifteen throat washings or suspensions of lung tissues from 12 patients having infections of the upper respiratory tract of various sorts, and (*c*) with serums from 4 normal persons. These experiments involved over 1,000 mice. Lungs from the control mice yielded on repeated occasions Nigg's mouse pneumonitis virus.[6] In contrast to the viruses of meningopneumonitis, ornithosis and psittacosis, this virus is not pathogenic for mice when inoculated intracerebrally. The pneumonia produced by it is readily identified by the formation of a "granular body" in the lungs of mice.[7]

These experiments indicate that the meningopneumotropic virus isolated from this patient is of human origin. This virus will be referred to as virus M (initial of the patient's name).

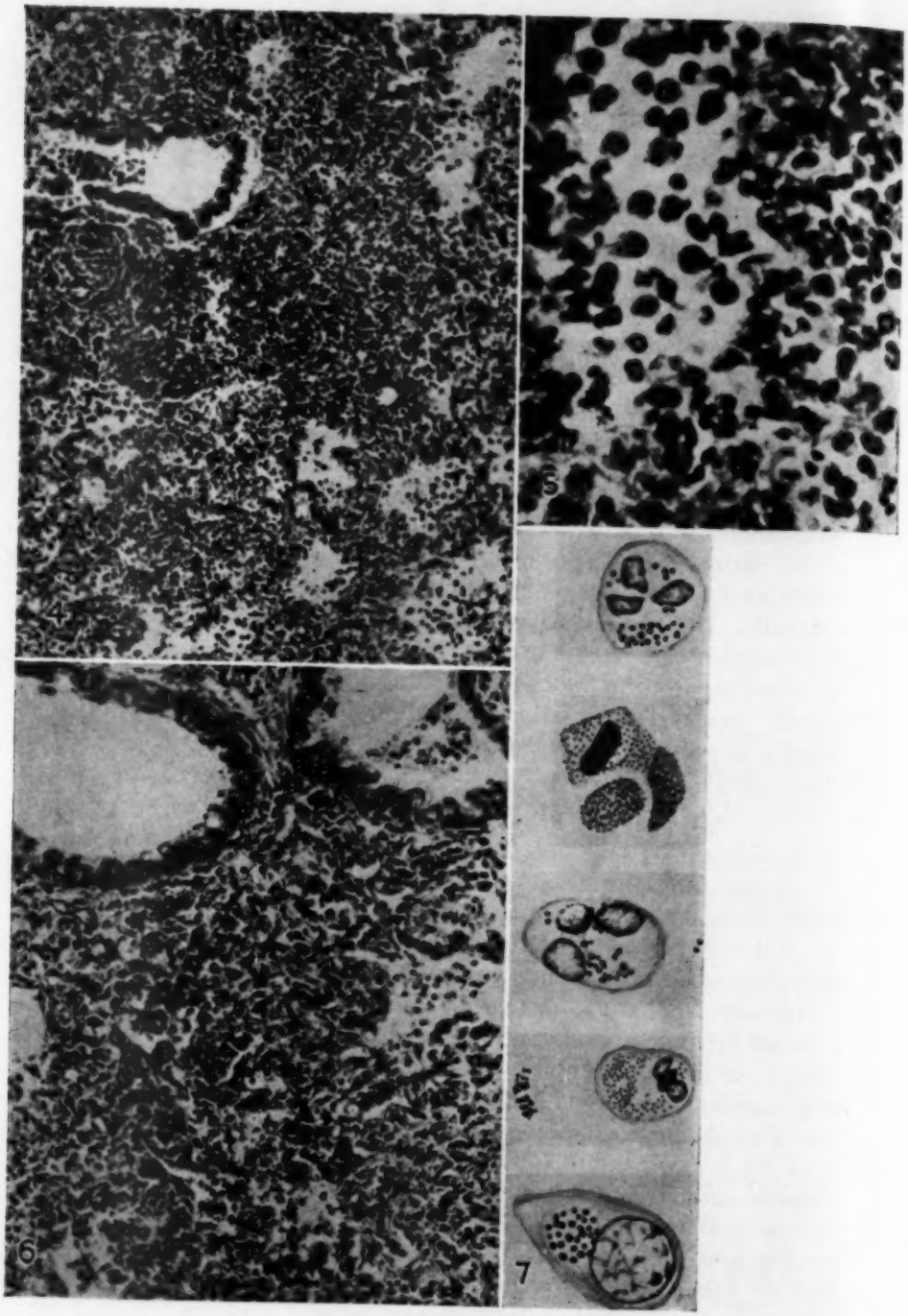
CHARACTERISTICS OF VIRUS M

Experimental Pneumonia Produced in Mice by Virus M.—On the second day after intranasal inoculation the lungs of the mice are glossy, congested and edematous. On successive days they become firm, red and red-gray and gray. There is no pleuritis or other change detectable on gross examination.

In mice dying at two to four days after inoculation the consolidations involve both alveoli and interstitial tissue, while the bronchi are either free or contain only scanty exudate (figs. 4 and 5). Both polymorphonuclear leukocytes and monocytes are abundant in the alveoli, the former being predominant; most leukocytes are neutrophilic (fig. 5); a few are eosinophilic. The alveolar lining cells are swollen. The alveolar septums are congested. There is only scant exudation of fluid with almost no fibrin. Many alveoli are filled with edema fluid. In some areas the alveolar septums are indistinct, and there is a pink-staining homogeneous or slightly fibrillary material in many alveoli.

6. Nigg, C.: Science **95**:49, 1942.

7. Furth, J., and deGara, P. F.: Proc. Soc. Exper. Biol. & Med. **56**:107, 1944.



Figures 4, 5, 6 and 7

(See legend on opposite page)

The consolidation is so massive in several places that it is not possible to distinguish septums from alveoli, both being densely infiltrated by leukocytes.

Elementary particles are seen in both monocytes and polymorphonuclear leukocytes, and also free (fig. 7). The elementary particles are spherical and tend to occur in small clumps. Formation of a large granular body such as that which characterizes histologically the virus of mouse pneumonitis does not occur. Most elementary particles of the latter virus are smaller than those of virus M. The elementary particles stained by Giemsa solution appear in the form of dark blue spherules. When stained with hematoxylin, they are pale, and their number is small; they do not take the Gram stain.

In animals dying at five days, the lumens of bronchi and bronchioles and of alveoli contain either edema fluid or exudate of polymorphonuclear leukocytes. The consolidation is patchy, mostly confluent; the alveoli contain many polymorphonuclear leukocytes and monocytes, and in places, serum precipitate.

At six days the alveolar structures are indistinct. Some alveoli contain pink-staining material; others, polymorphonuclear and mononuclear leukocytes. There is slight inflammation about the arteries, and the periarterial lymphatics are distended with polymorphonuclear leukocytes and monocytes.

At seven days (fig. 6) there is a predominance of mononuclear cells. In a few places, however, the alveolar exudate is composed mainly of polymorphonuclear leukocytes, suggesting progression of the pneumonic consolidation. In animals that die at about this time, there is congestion with alveolar hemorrhage and massive edema. This appears to be a terminal, possibly an agonal event, as it has not been noted in mice that had pneumonia and were killed at this time. The number of elementary particles in the lung is less than before. Lymphoid cells begin to accumulate about vessels. Large mononuclear cells proliferate in alveoli and interstitial spaces and appear in bronchi. Some of these are alveolar lining cells which have become swollen and desquamated. They are large in proportion to the alveolar lumens, so that a few can fill an air space.

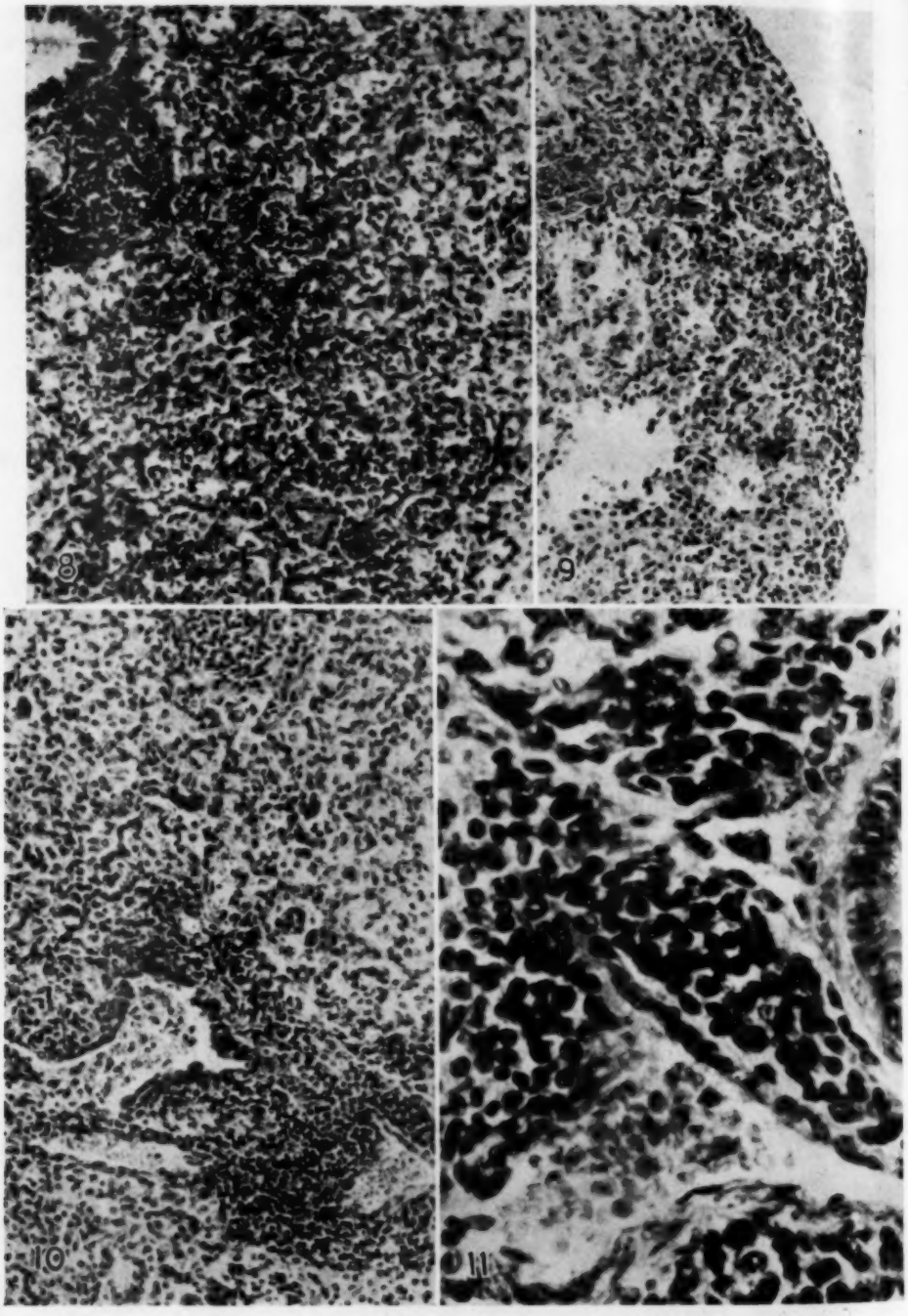
EXPLANATION OF FIGURES 4 TO 7

FIG. 4.—Section from the lung of a mouse dying three days after intranasal infection, showing the character of the consolidation. Hematoxylin-eosin; × 200.

FIG. 5.—Higher magnification of a field in figure 4, showing the exudate cells, mainly polymorphonuclear leukocytes, in an alveolus. Hematoxylin-eosin; × 300.

FIG. 6.—Section of lung of a mouse killed seven days after intranasal infection. It shows a relative increase of mononuclear cells and involvement of the interstitial tissue. Hematoxylin-eosin; × 200.

FIG. 7.—Elementary particles, free and in leukocytes, in section of consolidated lungs (drawings). The variability of the size of the elementary particles, their intracellular and extracellular location, and the tendency to form clumps are illustrated. Giemsa; × 1,000.



Figures 8, 9, 10 and 11 .

(See legend on opposite page)

The perivascular lymphoid infiltration increases in severity in the course of this pneumonia, in spite of partial resolution of alveolar exudate. Masses of normal-appearing small lymphocytes with few plasma cells and few large lymphocytes encircle almost all branches of the pulmonary vessels and extend as far as the hilus. The scantiness of these cells about bronchi is noteworthy.

At about ten days (fig. 8) the lumens of many bronchi and bronchioles are filled with leukocytes and cell debris, but there is no inflammation in the walls of bronchi. Pleurisy is never conspicuous, though in few areas the pleura is thickened by monocytes and other cells (fig. 9). Figures 10 to 15 illustrate the late phase of the inflammation (fourteen to eighteen days). There is massive perivascular lymphoid infiltration (figs. 11 and 13). The peribronchial spaces are free, but the lumens of bronchi are filled with large mononuclear cells (figs. 13 and 14). Massive lymphoid infiltration surrounds large vessels and extends into the hilus and mediastinal fatty tissue (fig. 15).

At seven to fourteen days the interstitial inflammation appears to be more conspicuous than at earlier phases of the disease, and thickening of the alveolar septums by large monocytes and fibroblast-like cells causes reduction of the alveolar lumens.

Thus, the inflammation produced by virus M is a predominantly alveolar consolidation with some interstitial pneumonia. Suppuration does not occur, and the alveolar framework does not break down, though it is often indistinct. Polymorphonuclear leukocytes are predominant in the early stages of pneumonia, when elementary particles are abundant, and monocytes are predominant in the later stages. A lymphoid "collar" persists about blood vessels, even after the elementary particles have disappeared and the pneumonia has been almost completely resolved.

Pathogenicity of Virus M for Mice.—Virus M when injected intracerebrally or instilled intranasally is pathogenic in all of four stocks of mice tested. It produces fatal meningoencephalitis with paralysis when injected by the intracerebral route, and pneumonia when in-

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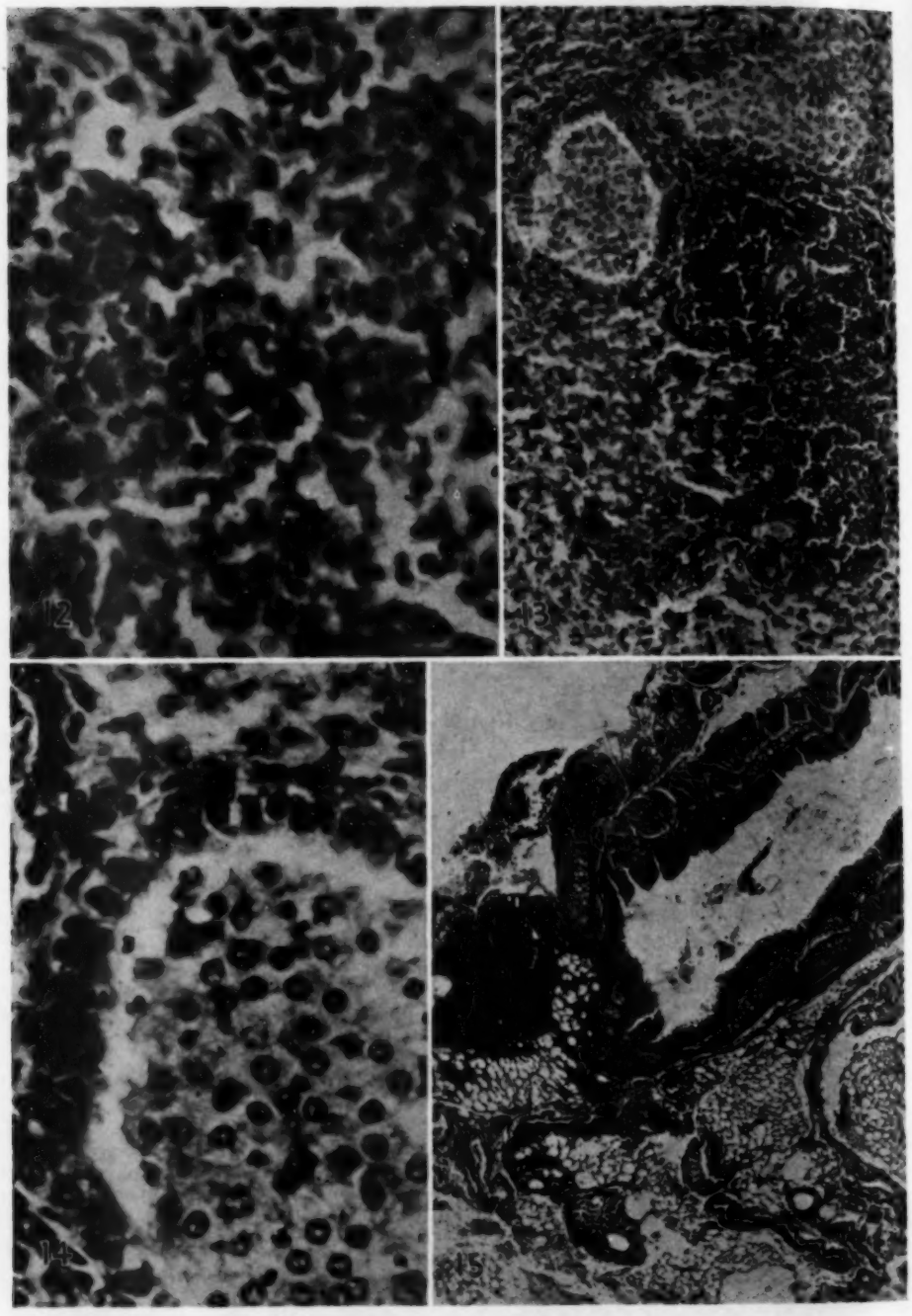
EXPLANATION OF FIGURES 8 TO 11

FIG. 8.—Section of lung of a mouse killed ten days after intranasal infection. There is advanced perivascular lymphoid infiltration. The bronchus is free, and the alveolar septums are indistinct. The inflammatory exudate is composed of lymphocytes, polymorphonuclear leukocytes and large monocytes. Hematoxylin-eosin; × 200.

FIG. 9.—Section of lung of a mouse dying fourteen days after intranasal infection. There is moderate pleurisy; the exudate cells are lymphocytes and monocytes. Hematoxylin-eosin; × 200.

FIG. 10.—Section of lung of a mouse killed fourteen days after intranasal infection. It shows perivascular lymphoid infiltration; the alveolar structure is indistinct. Most of the exudate cells are mononuclear. Hematoxylin-eosin; × 200.

FIG. 11.—Higher magnification (× 590) of the perivascular infiltration shown in figure 10.



Figures 12, 13, 14 and 15

(See legend on opposite page)

stilled into the nostrils. The disease remains localized to the organ inoculated, though the virus is present in the blood for at least fourteen days (see later section). The period of incubation and the duration of illness depend on the inoculating dose and the virulence of the virus; the latter may be so increased by frequent passages as to produce a fatal disease in two to three days in all infected mice. The passage virus is pathogenic for both young and adult mice. It is more pathogenic by the intracerebral than by the intranasal route; e.g., a 10^{-5} suspension of pneumonic lung is fatal to mice when injected intracerebrally but not when instilled intranasal.

Subcutaneous injection of pneumonic lung produced no illness in 10 mice.

Intratesticular injection of a concentrated suspension (10^{-1}) of pneumonic lung produced no generalized disease in 14 mice. In sections of the testes five days after the infection numerous elementary particles were seen in mononuclear and polymorphonuclear cells.

Intraperitoneal injections were made with a 10^{-1} suspension in 9 mice; 8 died within two to nine days. Of 9 mice that had received a 10^{-2} suspension, 3 died within the same period, while all 9 mice receiving the 10^{-3} suspension survived. At autopsy a small amount of serofibrinous exudate was found in the peritoneal cavity in 6 mice. On microscopic examination the livers showed disseminated focal hepatitis with miliary adscesses; numerous elementary particles were noted in these areas. By intranasal assays, the presence of the virus was demonstrated in livers, spleens, brains and lungs of mice from three to six days after the intraperitoneal injection. Intraperitoneal injection produces viremia persisting for at least twenty-one days.

Pathogenicity for Rats.—Intranasal instillations of a 10^{-1} suspension of infected mouse lung were made in 6 young and 7 adult albino rats. Four young rats died of pneumonia within two to four days. The 2 remaining rats, killed after four to five days, also had pneumonia and the characteristic elementary particles were seen in sections of the lungs. Intranasal subpassages were made four to five days after the intranasal instillations in 6 adult rats and 12 mice. They were

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EXPLANATION OF FIGURES 12 TO 15

FIG. 12.—Section of lung of a mouse killed fifteen days after intranasal infection. There is almost complete absence of polymorphonuclear leukocytes. The exudate cells are almost exclusively mononuclear. Hematoxylin-eosin; × 200.

FIG. 13.—Section of lung of a mouse killed eighteen days after intranasal infection. There is massive perivascular lymphoid infiltration, and the lumens of the bronchi are filled with large mononuclear cells. Hematoxylin-eosin; × 200.

FIG. 14.—Higher magnification (× 590) of a bronchus shown in figure 13. The alveolar lining cells are intact. The mononuclear cells in the bronchus originate probably from the alveolar exudate.

FIG. 15.—Massive lymphoid infiltration about mediastinal vessels; absence of inflammation of a main bronchus. Hematoxylin-eosin; × 150.

successful in 10 mice but in none of the rats. Three of the 7 adult rats infected, killed after four to seven days, had small areas of pulmonary consolidation with no elementary particles. Intranasal subpassages made after four to five days produced pneumonia in mice but not in rats. Two of 3 rats receiving a 10^{-1} suspension intracerebrally became paralyzed four days after the injection. Intracerebral subpassages made with brain suspensions failed in rats but were highly successful in mice.

Pathogenicity for Guinea Pigs.—Virus M is moderately pathogenic for guinea pigs by the intracerebral route and slightly by the intranasal route.

Additional Properties of Virus M.—The virus readily passes Berkefeld V filters, but much of it is withheld by N filters.

While these investigations were in progress, Heilman and Herrell[8] reported on the susceptibility to penicillin of psittacosis-like viruses. In our first two experiments 300 units of penicillin per day, which was sufficient to control experimental pneumococcic pneumonia of mice (MacLeod[9]), proved ineffective, but two additional experiments confirmed the work of Heilman and Herrell indicating that penicillin reduces both morbidity and mortality of virus M infections.

IMMUNOLOGIC INTERRELATIONSHIP AMONG THE PSITTACOSIS-LIKE VIRUSES

The range of pathogenicity, the appearance of elementary particles and the characteristics of the anatomic changes indicate that our virus M is a member of the group of psittacosis-like agents. A cross immunity study was undertaken to establish more closely the position of virus M.

Viruses Studied.—A strain of meningopneumonitis virus[2] was obtained from Dr. T. P. Magill, and a strain of mouse pneumonitis virus of Nigg[6] from Dr. F. L. Horsfall Jr. Several strains of mouse pneumonitis virus, including that named Br, were isolated in this laboratory[7]. Three strains of ornithosis virus were obtained from Dr. F. R. Heilman. The first, strain 804, was isolated by him from the spleen of a pigeon; the second, strain 295, from the sputum of a patient in whom pneumonia developed and who was exposed to pigeons; the third, strain 551, from a pigeon, by Dr. K. F. Meyer.

These experiments, which will not be detailed, indicate that there is complete, or almost complete, cross immunity between strain M and meningopneumonitis virus (strain MP) and no cross immunity between mouse pneumonitis virus and strain M. Ornithosis and strain M virus proved indistinguishable. The psittacosis virus produced

8. Heilman, F. R., and Herrell, W. E.: Proc. Staff Meet., Mayo Clinic. **19**:57 and 204, 1944.

9. MacLeod, C. M.: M. Clin. North America **27**:670, 1943.

strong immunity against itself and strain M and partial immunity against ornithosis virus; ornithosis virus protected against psittacosis virus almost completely but only partially against strain M.

Attempts to produce strong immunity with psittacosis virus met with little or no success. This agent proved fatal by the intraperitoneal route in doses as low as 10^{-9}.

After Dr. Eaton's virus SF17 had been received from him, its immunologic relation to strains M, ornithosis and psittacosis was tested, and it was found that strains M and ornithosis protect against Eaton's virus, but that strain SF does not protect against strains M and ornithosis. This is in agreement with the observation of Eaton and co-workers[4] that the ornithosis virus protects against his virus SF but that the reverse is not true.

COMMENT

Histologic Aspects of Human Psittacosis-like Pneumonia.—Classic psittacosis has been fully described by several pathologists. The first anatomic study of psittacosis by Eberth indicates that it is associated with a unique pulmonary consolidation (Foord[10]). There are only sketchy reports on the psittacosis-like pneumonia. So far as can be ascertained from a study of the literature, there is no essential difference between pneumonias produced by psittacosis and psittacosis-like viruses. They differ, however, from the common type of atypical pneumonia[11]. In the latter the consolidation is interstitial; in the former, alveolar. Both psittacosis and psittacosis-like pneumonias of man are essentially bronchopneumonias, sometimes with confluent consolidation, simulating lobar pneumonia. Large mononuclear cells are predominant in the alveoli. Some of these cells are variants of monocytes; others are lining cells of alveoli that have undergone hypertrophy and hyperplasia and have desquamated. Erythrocytes and occasionally fibrin are present in the alveoli in variable quantities. There is no marked pleural involvement, and bacteria are absent except when secondary infection supervenes. Reports of postmortem observation of changes in human psittacosis are few.

Wilson[12] described the anatomic findings in a fatal case of psittacosis as follows. The general picture is that of a septicemia. The lungs are congested and contain abundant serous exudate with much fibrin. The epithelial lining of the air vesicles and the bronchi is degenerated and desquamated. Thrombosis of the capillaries occurs, with necrosis of the walls of the air vesicles and secondary hemorrhage. The relative absence of polymorphonuclear leukocytes is striking and is consistent with the leukopenia. Polymorphonuclear leukocytes are present where

10. Foord, A. G.: Am. J. Clin. Path. **4**:247, 1934.

11. Primary Atypical Pneumonia Etiology Unknown, Official Statement, War Med. **2**:330, 1942.

12. Wilson, G. H.: J. Path. & Bact. **33**:957, 1930.

secondary pyogenic infection has occurred. In his experience the changes in the lung are different from those observed in the 1918 influenza epidemic. In psittacosis the consolidation is that of confluent lobular pneumonia with abundant fibrin formation, resembling lobar penumonia. In influenza it is essentially that of bronchopneumonia; there is capillary bronchitis, to which the pneumonia is related, and there is usually little fibrin; often there is extreme edema of the peribronchial and perivascular connective tissue. The exudate consists of enormous numbers of polymorphonuclear leukocytes, and suppuration may occur. In influenza the hemorrhage occurs without a tendency toward thrombosis in capillaries and apart from necrosis of lung tissues.

According to the description of MacLachlin, Permar and Rogers,[13] the most distinctive feature of psittacosis pneumonia is hypertrophy and hyperplasia of the cells lining the alveoli. However, these cells do not enter into the formation of the exudate. Autopsy, in their opinion, does not yield results characteristic enough to identify the disease without confirmatory epidemiologic data. The absence of interstitial inflammation and that of pleuritis are noteworthy.

Siegmund[14] described the changes in the lung as varying from simple edema to complete filling of alveoli with cellular exudate containing only little fibrin. The cells of the exudate in the alveoli are chiefly mononuclear phagocytes, while the number of polymorphonuclear leukocytes is small.

Lillie[15] analyzed the observations in the published records of autopsies in cases of psittacosis and those in 9 cases studied by him histologically. His conclusions follow: Consolidation in psittacosis is primarily focal or lobular and not especially related to the bronchioles. An apparent sequence of congestion and edema, red hepatization and gray hepatization may be observed, but all stages may be seen in the same case. Histologically fibrin, red cells, polymorphonuclear leukocytes and epithelial cells appear early in the exudate. Later fibrin disappears and large mononuclear leukocytes replace other cells. The alveolar epithelial cells undergo swelling, fatty degeneration and desquamation. They may be invaded by "Rickettsiae." Necrosis of septums is sometimes seen. Interstitial cellular infiltration is lacking, although an interstitial serous exudate may be present. Pleural reaction is of minor grade, if it is present at all. Focal necrosis in the liver occurred in only about one third of the cases, and granulomas were seen in only 1 case.

This description of psittacosis by Lillie and others matches the anatomic changes in pneumonias produced by psittacosis-like organisms, as well as those observed in our case.

McNaught[16] described the anatomic changes of a fatal type of virus pneumonia caused by a psittacosis-like agent that occurred on the Pacific coast as follows: The pneumonia is predominantly alveolar and not interstitial. The alveoli are filled with serum and a coarse network of fibrin containing large mononuclear leukocytes. The alveolar septums are thickened, owing to congestion, mononu-

13. MacLachlin, W. W. G.; Permar, H. H., and Rogers, C. A.: Ann. Int. Med. **4**:260, 1930.

14. Siegmund, H.: München. med. Wchnschr. **77**:223, 1930.

15. Lillie, R. D.: The Pathology of Psittacosis in Animals and the Distribution of Rickettsia psittaci in the Tissues of Man and Animals, National Institute of Health Bulletin 161, United States Treasury Department, Public Health Service, 1933.

16. McNaught, J. B.: California & West. Med. **59**:220, 1943.

clear infiltration and "swollen epithelium." The bronchi contain desquamated epithelium, fibrin, mononuclear cells and many polymorphonuclear lekocytes. There is only slight inflammation in their walls.

A new strain of psittacosis-like virus has recently been isolated from cases of epidemic pneumonitis in the Bayou region of Louisiana by Olson and Larson[17]. It produces a disease which resembles psittacosis[18], but it differs from the psittacosis-like viruses in that it produces a fatal infection in guinea pigs, as well as in mice, and in the latter species also after intramuscular infection.

This survey of the histopathologic aspects of pneumonias produced by psittacosis and related viruses indicates that they resemble each other but differ from other known virus pneumonias, including "primary atypical pneumonia." The latter is essentially an interstitial inflammation (Golden[19]). No definite anatomic criteria are known on which a subdivision of the psittacosis-like pneumonias could be based.

Interrelationship of Psittacosis-like Viruses.—The experiments described indicate that the anatomic changes produced by virus M and its immunologic properties are essentially those of the meningopneumonitis virus of Francis and Magill[2], first isolated by inoculating ferrets with throat washings from a patient suspected of having epidemic influenza. After subpassages in ferrets, the virus was transmitted to mice and maintained in this species. It produces fatal pneumonia after intranasal instillation, and fatal meningitis after intracerebral inoculation. Paralysis of extremities occurs in a moderate percentage of mice after intraperitoneal or subcutaneous administration of the virus, while the remainder become immune to intracerebral reinfection but not to intranasal reinfection. Francis and Magill found it impossible to decide whether the virus was of ferret or of human origin. They recognized the resemblance of this virus to those of psittacosis and lymphogranuloma venereum, and differentiated it from psittacosis chiefly by the absence of hepatic necrosis, produced by the latter in mice and guinea pigs, and from the virus of lymphogranuloma venereum by the mildness or the absence of pulmonary lesions in mice infected with the latter virus. The virus of Francis and Magill is usually referred to as of ferret origin, but in the light of our investigations its human origin seems more probable. The pathogenicity of this virus for man was inadvertently proved by a laboratory infection[20].

17. Olson, B. J., and Larson, C. L.: Pub. Health Rep. **59**:1373, 1944.
18. Binford, C. H., and Hauser, G. H.: Pub. Health **59**:1363, 1944.
19. Golden, A.: Arch. Path. **38**:187, 1944.
20. Meiklejohn, G.; Beck, M. D., and Eaton, M. D.: J. Clin. Investigation **23**:167, 1944.

The term "ornithosis" was subsequently introduced by Meyer, Eddie and Yanamura[3a] who proposed to limit the term "psittacosis" to human infections proved to be caused by psittacine birds and to designate those due to contact with other birds as ornithosis. This term found wide acceptance in spite of the fact that viruses so named do not seem to differ from the meningopneumonitis virus of Francis and Magill.

Eaton[4] isolated from patients with atypical pneumonia the "human pneumonitis virus," which exhibits minor differences in comparison with the meningopneumonitis virus of Francis and Magill[2].

According to most investigators, psittacosis virus stands apart from psittacosis-like viruses. It is highly pathogenic for mice by the intraperitoneal route, and immunizes inconsistently against itself and still less against psittacosis-like viruses. After intraperitoneal infection it produces fibrinopurulent exudate persisting for weeks or months. From such exudates we recovered the virus as late as seventy-one days after infection. Animals with psittacotic peritonitis may die weeks or months after infection. This illustrates the difficulty of producing active immunity with living psittacosis virus, and the few attempts at immunization made with formaldehyde-killed virus have likewise been unsuccessful.

Meiklejon, Beck and Eaton[20] recently reviewed 10 human cases of atypical pneumonia produced by psittacosis-like viruses. One of the infections, the mildest, was contracted in the laboratory and was caused by a strain of meningopneumonitis virus which seemed indistinguishable from pigeon ornithosis virus. From 5 patients, 3 of whom had fatal infections, the "human pneumonitis virus" was isolated and in 4 patients, 2 of whom died of their disease, the ornithosis virus was demonstrated. These authors expressed the belief that the close similarity of the meningopneumonitis and the ornithosis virus suggests an original avian source of the former and pointed out that it is still undetermined whether "human pneumonitis virus" strain SF of Eaton is of avian or nonavian origin. Beck, Eaton and O'Donnell[21] concluded that the psittacosis-like viruses can be classified in three groups: psittacosis, ornithosis and human pneumonitis. They expressed the belief that the meningopneumonitis virus is of ferret origin.

In the course of studies of atypical pneumonia, Dingle and coworkers[22] have isolated viruses from 6 patients with nonfatal infections of the upper respiratory tract. They recovered the "virus of meningopneumonitis or ornithosis" from the sputums of 2 patients with atypical pneumonia and from throat washings of 4 patients, 2 with

21. Beck, M. D.; Eaton, M. D., and O'Donnell, R.: J. Exper. Med. **79**:65, 1944.
22 Dingle, J. H., and others: Am. J. Hyg. **39**:167 and 269, 1944.

atypical pneumonia, 1 with bronchitis resembling atypical pneumonia, and 1 with an acute disease of the respiratory tract in which sinusitis was the prominent feature. They expressed the belief that these viruses are widespread and can exist also as saprophytes.

The fact that in a single case of primary atypical pneumonia virus M was isolated from a bacteriologically sterile consolidated lung in each of three successive attempts to infect mice, and in one, by inoculating a suspension of frozen lung directly into egg embryos, indicates that this virus was present in the human lung. Extensive control experiments aimed at recovering a similar virus from the lungs of normal mice of the same stocks or from material from normal and diseased persons, involving over 1,000 mice, failed to yield a similar virus. These experiments indicate that psittacosis-like viruses are not common saprophytes of the nasopharynx of man or of the lungs of mice. The virus of the mouse pneumonitis of Nigg[6], on the contrary, has a saprophytic existence. It is readily distinguished from the psittacosis-like viruses (*a*) by its lack of meningotropism, (*b*) by the failure of cross protection with the psittacosis-like virus, (*c*) by the characteristic "granular bodies," composed of elementary particles, formed in the consolidated lungs of mice.

The elementary particles of meningopneumonitis virus and those of strain M are indistinguishable as are all other characteristics of these viruses, and it seems that the ornithosis and meningopneumonitis viruses are essentially alike.

Eaton's virus named human pneumonitis[4] has some individual immunologic features, but these are no more marked than those of different strains of pneumococci, and the designating of this virus by a new name may not be justified.

The paper of Zichis and Shaughnessy[23] indicates that their "Illinois virus," too, possesses distinctive immunologic characteristics. Since this distinction is but slight, it seems desirable to regard this as another (Illinois) strain of psittacosis-like viruses.

The different strains of psittacosis-like viruses are differentiated from one another on the following basis: (*a*) source, (*b*) virulence in mice following intraperitoneal injection, (*c*) virulence in pigeons and other birds following intracerebral inoculation, (*d*) cross immunity by intraperitoneal immunization and intracerebral reinjection and (*e*) latency in mice or birds.

The source alone is not a trustworthy basis for differentiating viruses.

When injected intraperitoneally: (*a*) the psittacosis virus kills the mice, and at autopsy foci of necrosis are seen in liver and spleen; (*b*) the human pneumonitis virus produces no damage (Beck and as-

23. Zichis, J., and Shaughnessy, H. J.: Science **102**:302, 1945.

sociates[21]); (*c*) the ornithosis and meningopneumonitis viruses are of variable virulence but do not produce the hepatic damage elicited by the psittacosis virus. These differences can be explained by assuming that the "human pneumonitis virus" is the least virulent and the psittacosis virus the most virulent member of the group. As Beck and co-workers[21] have shown, the "human pneumonitis virus" is the only member of the group which does not invariably produce fatal meningoencephalitis when injected intracerebrally into pigeons.

Pinkerton and Moragues[24] compared the intracranial virulence for pigeons and mice, and the intraperitoneal virulence for mice, of the following viruses: meningopneumonitis, human pneumonitis, psittacosis of pigeon origin and psittacosis of parrot origin. They arrived at the conclusions that psittacosis virus of pigeon origin and meningopneumonitis virus are probably biologically modified strains of typical parrot psittacosis virus and that "human pneumonitis" virus is closely related to this group.

The intricate relationship among these viruses is indicated by cross immunity tests. The human pneumonitis virus is stated to immunize only against itself, whereas the ornithosis and meningopneumonitis viruses immunize against all other viruses, including "human pneumonitis." Recently Hilleman and Gordon[25] found that meningopneumonitis virus immune serum produced in roosters neutralizes both the meningopneumonitis and the ornithosis virus but not the viruses of mouse pneumonitis, feline pneumonitis (Baker[26]) and lymphogranuloma venereum. This confirms other evidence that these three viruses are distinct.

Thus it seems that the viruses named meningopneumonitis, ornithosis and human pneumonitis are variants of the same type of virus. It is possible that, if there are minor antigenic differences among them, these are obscured by the common antigenic components.

Smadel[27] has arrived at essentially the same conclusion, finding no sharp distinction between meningopneumonitis, psittacosis and ornithosis viruses, regarding them all as strains of one virus.

Nomenclature.—Between filter-passing ultramicroscopic agents and common micro-organisms are the disease-producing agents which are small but visible under the microscope and seem to require for their multiplication living cells. These agents could be named cytomicrobes. Cytomicrobes can be subdivided into those which require an arthropod vector as intermediate host (the Rickettsiae) and those which do not (the agents of lymphogranuloma venereum and psitta-

24. Pinkerton, H., and Moragues, V.: J. Exper. Med. **75**:575, 1942.
25. Hilleman, M. R., and Gordon, F. B.: Science **98**:347, 1943.
26. Baker, J. A.: Science **96**:475, 1942; J. Exper. Med. **79**:159, 1944.
27. Smadel, J. E.: J. Clin. Investigation **22**:57, 1943.

cosis and related organisms). The antigenic relation of the virus of lymphogranuloma venereum to psittacosis-like viruses is known (Rake, Eaton and Shaffer[28]).

The interrelationship of the known psittacosis-like viruses requires further study before distinct names such as meningopneumonitis, ornithosis and human pneumonitis are accepted for types of agents which are alike in essential properties.

SUMMARY

From the lung of a patient dying of atypical pneumonia a psittacosis-like virus was isolated. Control experiments indicate that this virus is neither a saprophyte in mice nor is it an inhabitant of the throat or the lungs of the normal human being.

The histologic changes in the lung of the patient were similar to those produced by the viruses of the psittacosis group, and a survey of the literature disclosed no marked differences between psittacosis and psittacosis-like pneumonias.

The experimental pneumonia produced in mice by this virus was predominantly alveolar, with some interstitial consolidation. The cells of the exudate were polymorphonuclear leukocytes and large mononuclear cells. The inflammation was characterized by the presence of abundant spherical elementary particles, free and within the cells of the exudate, during the first two weeks of illness. During the second week a perivascular lymphoid infiltration developed, which gradually increased in intensity and persisted after disappearance of the elementary particles and resolution of the alveolar exudate.

The virus isolated by us is highly pathogenic for mice by the intracerebral route, almost as pathogenic by the intranasal route and is but slightly pathogenic by other routes. It is present in the blood of infected mice for at least three weeks. It is slightly pathogenic for rats and guinea pigs and is moderately suceptible to penicillin.

Immunologic studies have shown that the psittacosis-like agents variously designated as meningopneumonitis, ornithosis and human pneumonitis either are indistinguishable or exhibit only minor antigenic differences. Hence it is proposed to name them all psittacosis-like viruses. Since these agents seem to require living cells for multiplication but are visible, as are rickettsias, it is proposed to group them under the collective term "cytomicrobes."

28. Rake, G.; Eaton, M. D., and Shaffer, M. F.: Proc. Soc. Exper. Biol. & Med. **48**:528, 1941.

SYNERGISTIC NECROTIZING ACTION OF URETHANE AND CHLOROFORM

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IN PRELIMINARY DETERMINATIONS of the human tolerance of chemotherapeutic agents used in treating patients for inoperable cancer, fatalities cannot always be avoided; and arsenicals, benzene and many other substances have produced fatal human lesions which later served to define the extent of therapeutic usefulness. The recent reports[1] concerning urethane (ethyl carbamate) as used clinically in cancer chemotherapy lacked any warning of dangerous side effects other than leukopenia in spite of the fact that urethane is a well known drug with toxic and anesthetic properties[1g].

Three deaths occurred unexpectedly in a series of elderly patients treated with urethane for widespread prostatic carcinoma. A preliminary report of these cases has been published by Huggins, Yu and Jones[2] and a detailed clinical report will be published elsewhere by these authors:

The first patient, P.C., was moribund and in congestive heart failure when admitted to the hospital. He was digitalized successfully. During the next eleven days he was given urethane in chloroform water U.S.P. orally,[3] receiving in all 126 Gm. of urethane and 4 Gm. of

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Part of the work described in this paper was done under a contract between the Medical Division, Chemical Corps, United States Army and the University of Chicago Toxicity Laboratory. Under the terms of the contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.

1. (*a*) Patterson, E.; Haddow, A.; ApThomas, I., and Watkinson, J. M.: Lancet **1**:677, 1946. (*b*) Moeschlin, S.: Helvet. med. acta **14**:279, 1947. (*c*) Shulze, E.: Deutche med. Wchnschr. **72**:153, 1947. (*d*) Schulze, E.; Fritze, E., and Muller, H. H.: ibid. **72**:371, 1947. (*e*) Maringer, S.: Schweiz. med. Wchnschr. **77**:114, 1947. (*f*) Webster, J. J.: J.A.M.A. **135**:901, 1947. (*g*) Hirschboeck, J. S.; Lindert, M.C.F.; Chase, J., and Calcy, T. L.: ibid. **136**:90, 1948.

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3. The urethane was administered after the prescription of Patterson and coworkers[1a] urethane, 1 Gm.; orange syrup U.S.P., 1.5 cc., and chloroform water U.S.P. to make 15. cc. When 6. Gm. or more of urethane was given daily, the urethane was increased in amount, but the rest of the prescription was kept constant.

chloroform. This treatment was stopped one week before his death because of increasing lethargy, disorientation, anorexia and vomiting. During treatment he lost 7 Kg. Congestive cardiac failure and death followed the sudden onset of a gallop cardiac rhythm. The second patient, M.S., was treated with urethane in chloroform water U.S.P. by mouth for thirty-five days, receiving in this time 356 Gm. of urethane and 12 Gm. of chloroform. Treatment was discontinued because the white blood cell count fell from 8,400 to 4,300 per cubic millimeter. After therapy was stopped, the white cell count continued to fall to 2,100 per cubic millimeter, and icterus gradually developed. Icterus was intense for the last four days before his death, which occurred twelve days after cessation of treatment. The third patient, W.S., was given increasing doses of urethane in chloroform water U.S.P. for fourteen days and then maintained on 12 Gm. of urethane per day for seven days, receiving in all 134 Gm. of urethane and 7.3 Gm. of chloroform. During the last week of treatment he had severe bronchitis and became progressively weaker. Treatment was terminated when his white blood cell count, which was 6,800 per cubic millimeter on admission, was found to be 2,000 per cubic millimeter, 85 per cent being polymorphonuclear leukocytes. During the next five days he gradually became jaundiced and had bouts of severe nausea, anorexia and vomiting. After three days of intense icterus, he became comatose and died, about four weeks after treatment with urethane was started.

At necropsy, these men all had primary prostatic carcinoma with metastases in the regional lmyph nodes, the bones and the lungs. Histologically, the tumors were exceptionally well differentiated adenocarcinoma with little mitotic activity. In addition to terminal bronchopneumonia and bronchitis, all the patients had marked depletion of the lymphocytes of the lymph nodes and spleen and atrophy of the myelogenous tissue of the bone marrow. The marrow of the third patient showed throughout scattered nuclear debris and pyknotic degenerating forms of mature leukocytes.

Pathologically, the hepatic changes were the most outstanding. The liver of the first case patient (necropsy by C. Harold Steffee) weighed 910 Gm. Histologically, the cords around the central veins were irregular and tortuous. The hepatic cells had irregularly sized nuclei, double nuclei or pyknotic nuclei, as well as large amounts of lipochrome. The liver of the second patient was small but weighed 1,600 Gm. The lobules were brown and had red central areas surrounded by yellow zones. The intrahepatic portal veins contained recent thrombi. Histologically, this liver had areas of marked atrophy and sinusoidal dilation and occasional foci where centrally only the collagenous framework remained. In most areas, the central hepatic cells had macronuclei, double, pyknotic, fading or no nuclei and

fine droplet fat in the cytoplasm. The liver of the third patient weighed 1,350 Gm. The capsular surface was finely granular and dark brown. The markings of the freshly cut surfaces appeared typically "nutmeg." Histologically, the hepatic lobules were the site of central necrosis which extended through the midzonal areas, involving up to 75 per cent of each lobule, and was ringed by hemorrhage and a zone of degenerating hepatic cells extending to within six to eight cells of the portal connective tissue (fig. 5).

From the clinical observations and the results of the necropsies it appeared that hepatic injury had played an important role in the deaths of at least the last 2 patients. The widespread hepatic necrosis in the third patient was typical of chloroform poisoning. The largest amount of chloroform ingested (12 Gm. in thirty-five days) was, in our opinion, too small in comparison with the reported fatal human single ingested dose of 60 Gm.[4] to account for these deaths. It seemed possible, however, that since urethane is also a hepatotoxin[5], sublethal amounts of chloroform might act synergistically with urethane to produce a fatal hepatic lesion. This possibility was tested in rabbits, as follows:

MATERIAL AND METHODS

Two experiments were performed. In all, 71 rabbits, averaging 2.5 Kg. in weight, were used. In the first experiment, employing 53 rabbits, the lethal doses of urethane and chloroform were roughly determined when each was given alone and when the two were given in combination. The urethane, in 4 per cent aqueous solution, was administered intraperitoneally daily for nine consecutive days. The chloroform, in peanut oil, was given subcutaneously once, on the fourth day of injection of urethane. The dosages were on a per kilogram basis. The numbers of rabbits and the various doses of each drug used in this experiment are recorded in table I. All animals were submitted to pathologic examination at death or when killed at the end of the urethane treatment, and sections were made of the organs for histologic study.

In the second experiment 18 rabbits were divided into three groups of 6 animals each. The animals of the first group were given 400 mg. of urethane per kilogram intraperitoneally daily for four days and then killed in pairs at three eighteen hour intervals. The rabbits of the second group were given 0.2 cc. of chloroform per kilogram subcutaneously, in peanut oil, and then killed in pairs as in the first group. The animals of the third group received 400 mg. of urethane per kilogram intraperitoneally for four days and then were given 0.2 cc. per kilogram of chloroform in peanut oil subcutaneously. They were then killed according to the schedule used in the other groups except that 3 rabbits died thirty, forty-two and fifty-four hours after receiving the dose of chloroform. Necropsies of all animals in this experiment were made and sections of the organs prepared as in the first experiment.

RESULTS

The data from the first experiment, namely, the mortality and the average period of survival after urethane or chloroform had been given, alone, and after the two had been given, are summarized in table 1. A summary of the histopathologic effects observed in the second experiment has been made in table 2.

4. McNally, W. D.: Medical Jurisprudence and Toxicology, Philadelphia, W. B. Saunders Company, 1939.

5. Doljanski, L., and Rosin, A.: Am. J. Path. **20**: 945, 1944.

Mortality and Histopathologic Effects of Various Daily Doses of Urethane—Only 1 of 2 rabbits that received 1,000 mg. of urethane per kilogram daily died, six and one-half days after the treatment was started. All other animals that received urethane alone survived. Postmortem cytologic changes in the dead rabbit were too advanced for accurate determination of the effect of urethane, but in the liver there was evidence of central karyolysis and necrosis and in the lymphatic organs there were signs of progressive destruction of lymphocytes and atrophy. The tissues of the surviving animals revealed

TABLE 1.—*Mortality Data and Average Length of Survival of 50 Rabbits Given Various Amounts of Urethane (Intraperitoneally) and/or Chloroform (Subcutaneously)*

Urethane, Mg. per Kg. Daily	Chloroform Cc. per Kg. on the Fourth Day of Administration of Urethane					
	0	0.05	0.1	0.2	0.6	1.2
0		0/1*	0/2	1/2 (58 hr.)†	2/2 (48 hr.)	2/2 (44 hr.)
200	0/4	0/2	0/2	1/2 (60 hr.)	2/2 (42 hr.)	2/2 (30 hr.)
400	0/4	0/2	2/2 (60 hr.)	4/4 (46 hr.)	2/2 (30 hr.)	2/2 (16 hr.)
800	0/2	2/2 (48 hr.)	2/2 (37 hr.)	2/2 (22 hr.)	2/2 (20 hr.)	2/2 (24 hr.)

*The numerator equals the number dead; the denominator the number of rabbits used.
†The average number of hours the rabbits lived after receiving the injection of chloroform.

TABLE 2.—*Summary of the Pathologic Effects of Urethane and Chloroform, Alone and Combined, on the Liver, the Spleen and the Intestinal Mucosa of Rabbits*

Hours After Administration of Chloroform	Urethane, 400 Mg. per Kilogram daily			Chloroform 0.2 Cc. per Kilogram			Urethane and Chloroform Same Doses in Combination		
	Liver	Spleen	Intestinal Mucosa	Liver	Spleen	Intestinal Mucosa	Liver	Spleen	Intestinal Mucosa
18	0	0	0	Central eosinophilia	0	0	Necrosis 60%	Lymphocytic rhexis	0
36	0	0	0	Central fatty change	0	0	Necrosis 90%	Lymphoid and myeloid atrophy	Moderate to extensive pyknosis
54	0	0	0		0	0	Necrosis 75% Repair	Marked atrophy	0

no changes other than atrophy of the lymphatic organs, which was roughly proportional to the dose.

Mortality and Histopathologic Effects of Various Single Subcutaneous Doses of Chloroform—Both rabbits given 1.2 cc. per kilogram of chloroform in peanut oil died forty-four hours after the injection. Both animals given 0.6 cc. per kilogram died forty-seven and forty-nine hours later, respectively. One of the 2 animals given 0.2 cc. per kilogram died fifty-eight hours after injection; the other survived. The rabbits given the other two smaller doses survived. Histo-

logically, those rabbits that died, irrespective of the size of the dose or the length of survival, had central necrosis of hepatic lobules, involving the major portion of the lobule, and loss of lymphocytes by rhexis in the spleen, the thymus and the lymph nodes. The survivors had no pathologic changes other than aspermia with or without focal testicular tubular atrophy.

Mortality and Histopathologic Changes After Various Daily Doses of Urethane Combined with Increasing Single Doses of Chloroform.—200 Mg. of Urethane per Kilogram: This dose produced little change in the mortality or the length of time the animals survived after receiving the various increments of chloroform. Both rabbits given 1.2 cc. per kilogram of chloroform died, surviving on the average, thirty hours. The pair of animals that received 0.6 cc. per kilogram survived, on the average, forty-two hours. Only 1 of the 2 rabbits given 0.2 cc. per kilogram died, surviving sixty hours. The other animals survived until killed. Histologically, the changes in the animals that died were similar to those seen in the animals that died after receiving chloroform alone. The only change found in occasional ones of those that survived till killed was testicular atrophy.

400 Mg. of Urethane per Kilogram: This dose enhanced the effectiveness of the chloroform. Both rabbits given 1.2 cc. per kilogram of chloroform died, surviving only sixteen hours. Both animals receiving 0.6 cc. per kilogram died after an average survival of thirty hours. All 4 rabbits given 0.2 cc. per kilogram died, surviving, on the average, forty-six hours. The pair that received 0.1 cc. per kilogram died in about sixty hours. The 2 rabbits given 0.05 cc. per kilogram survived till killed. Histologically, in these 2 rabbits, edema and loss of fat in the bone marrow were the only pathologic changes found. In the other animals there were: central necrosis of hepatic lobules, involving 80 to 90 per cent of each lobule; atrophy of the lymphoid follicles and red pulp of the spleen; atrophy of the lymph nodes, thymus and intestinal lymphoid follicles; atrophy of the testicular tubular epithelium.

800 Mg. of Urethane per Kilogram: This daily dose of urethane further increased the toxicity of the various doses of chloroform. None of the animals survived indefinitely. The average length of survival was: twenty-four hours after 1.2 cc. of chloroform per kilogram; twenty hours after 0.6 cc. per kilogram; twenty-two hours after 0.2 cc. per kilogram; thirty-seven hours after 0.1 cc. per kilogram; forty-eight hours after 0.05 cc. per kilogram. Histologically, the changes were similar to, but more marked than the changes seen in the preceding group. In addition, karyolysis of the mucosal cells of the crypts of Lieberkühn in the jejunum was present. The central necrosis of hepatic lobules in the animals given 0.05 cc. per kilogram involved only about 30 per cent of each lobule.

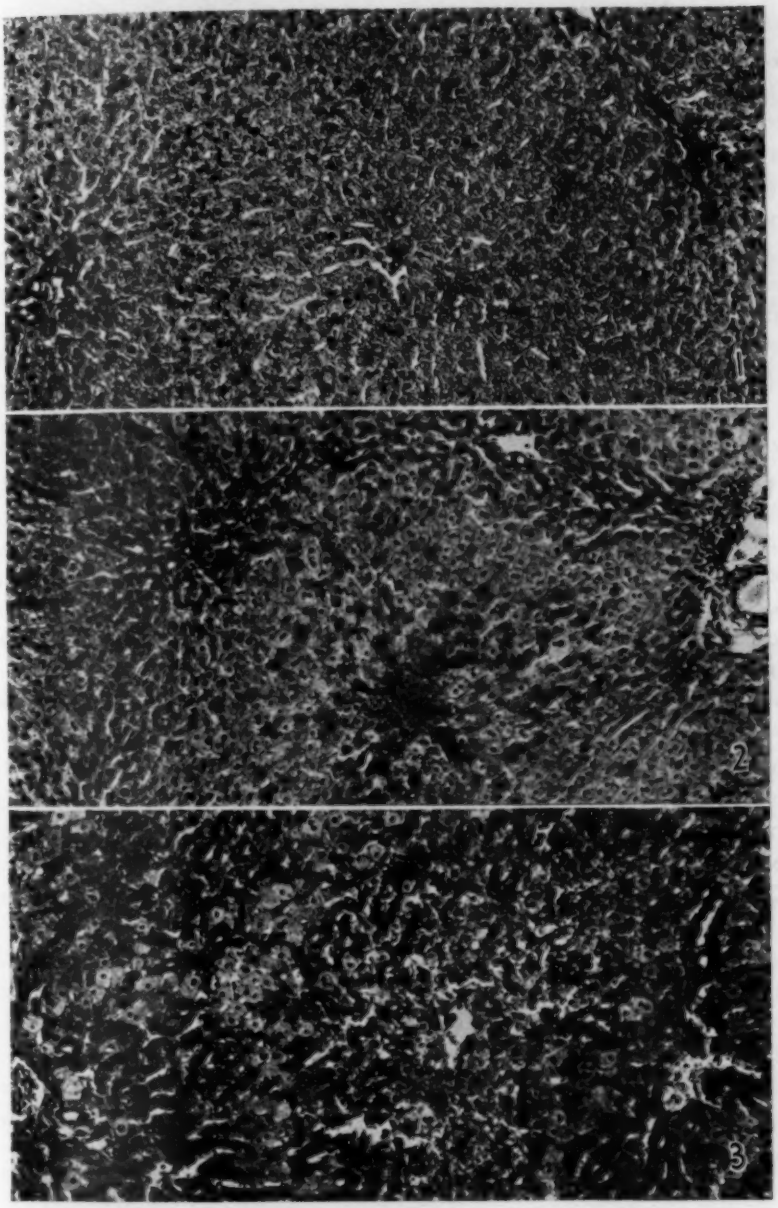

FIG. 1.—Liver of a rabbit examined eighteen hours after the administration of the last of four daily doses of 400 mg. of urethane per kilogram; hematoxylin and eosin stain; × 100.

FIG. 2.—Liver of a rabbit eighteen hours after the administration of 0.2 cc. or chloroform per kilogram, showing eosinophilic central cells and a surrounding zone of fatty change; hematoxylin and eosin stain; × 100.

FIG. 3.—Liver of a rabbit eighteen hours after these dosages of the two drugs had been administered in combination, showing extensive central necrosis; hematoxylin and eosin stain; × 100.

Pathologic Effects of Urethane and Chloroform When Each Was Given Alone and When the Two Were Given, in Rabbits Killed at Eighteen Hour Intervals (*table* 2).—After four daily doses of 400 mg. of urethane per kilogram alone no histologic changes were seen in any organs regardless of the period that elapsed before the animal was killed. The normal appearance of the liver in these animals is seen in figure 1.

After the single dose of 0.2 cc. of chloroform per kilogram, the liver was the only organ to have pathologic changes. Eighteen hours after injection, the hepatic cells of the central portion were eosinophilic and had pyknotic and occasionally disrupted nuclei (fig. 2). After thirty-six hours, fine and large droplet fat was present in these central hepatic cells, but the parenchymatous degenerative changes were gone. Fifty-four hours after injection no changes were found.

When both substances were given, all the animals at the various intervals had central hepatic necrosis. At eighteen hours a little more than half of each hepatic lobule was necrotic. This coagulation necrosis was rimmed by a zone of eosinophilic cells with pyknotic nuclei undergoing rhexis (fig. 3). By thirty-six hours the coagulation necrosis had extended to include the entire lobule except for a thin rim of hepatic portal cells. This zone, six to eight cells thick, contained fat in fine droplets (fig. 4). By fifty-four hours cellular regeneration was occurring at the periphery of the necrotic foci. The zone of necrosis then involved three quarters of the lobule. In the spleen, lymphocytic debris was present in lymphoid follicles in the animals killed at eighteen hours. Those dying or killed around thirty-six hours had continued rhexis of lymphocytes and marked atrophy of the lymphoid follicles. The myeloid cells of the red pulp were decreased to about half the normal number. By fifty-four hours the trabecular arteries had only a thin, sparse collar of lymphocytes and the pulp was almost completely devoid of polymorphonuclear leukocytes. In the duodenum and jejunum, no changes were seen in eighteen hours, but by thirty-six hours mucosal nuclear pyknosis and rhexis involved about half of the epithelium of the crypts. By fifty-four hours most of this change was gone. Testicular tubular atrophy and aspermia were present at thirty-six and fifty-four hours.

COMMENT

It would seem from these experiments that the toxicity of either urethane or chloroform is enhanced by the addition of the other drug. From the data in table 1, the dose of chloroform that can be expected to kill about 50 per cent of the rabbits appears to be about 0.2 cc. per kilogram. The lethal dose of urethane that can be expected to kill 50 per cent of rabbits after repeated intraperitoneal injections is about 1,000 mg. per kilogram. When slightly less than half of the L.D. 50

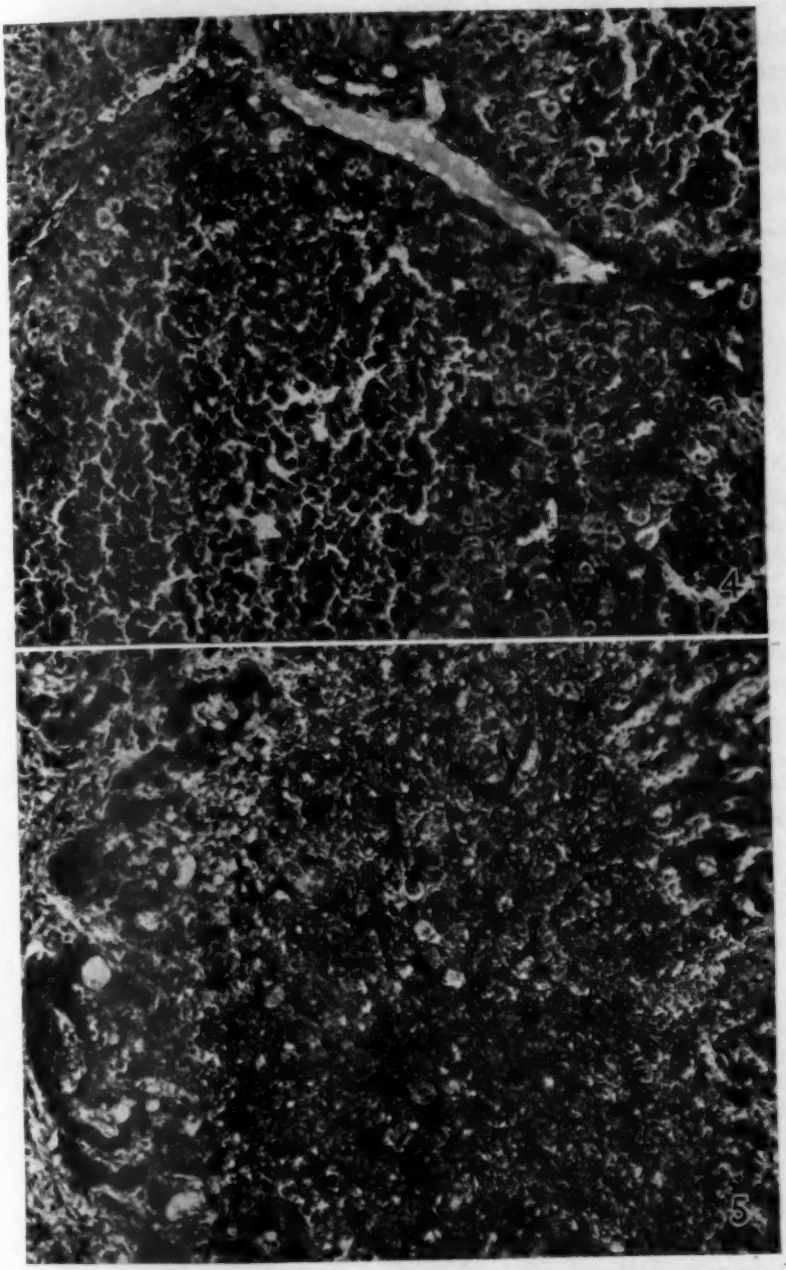

FIG. 4.—Liver of a rabbit examined thirty-six hours after both urethane (four daily doses of 400 mg. per kilogram) and chloroform (0.2 cc. per kilogram) had been administered, showing necrosis involving almost all of a lobule; hematoxylin and eosin; × 100.

FIG. 5.—Liver of the third patient as it appeared five days after the last administration of both drugs, four weeks after treatment was begun; hematoxylin and eosin; × 100. It shows central necrosis with hemorrhage similar to that seen in the rabbit's liver (fig. 4).

for repeated injections of urethane (400 mg. per kilogram) was combined with about half of the L.D. 50 of chloroform (0.1 cc. per kilogram), both animals that received this treatment died, an indication of a distinctly additive synergism. The observation that rats anesthetized with urethane during sublethal roentgen irradiation died as though they had received a lethal exposure to roentgen rays[6] is undoubtedly another example of the augmentation of the effect of an injurious agent by urethane. In the second experiment the mutual enhancement of the histopathologic effects of the two drugs is obvious (table 2 and figs. 1 to 3).

No attempt was made in these experiments to duplicate the actual dosage schedule of the men studied clinically. For the sake of convenience, the simpler means of dosage was chosen. The demonstration of the fact that a sublethal amount of chloroform acts synergistically with urethane to cause fatal hepatic necrosis in animals would seem to indicate the basis for the hepatic injury which played a role in the deaths of at least 2 of the 3 patients described. The similarity of the histopathologic changes in the third human case when they are compared with those of the rabbits given both drugs is striking (figs. 4 and 5). The cause of the fatal cardiac arrhythmia in the first case is, of course, not known. It is possible, however, that chloroform, which also has a toxic effect on the myocardium, may have enhanced the cardiotoxic properties of the digitalis which this patient was receiving.

These experiments demonstrated again that chloroform should not be used in conjunction with other medicinal preparations. They also support the thesis that before inoperable cancer of moribund men is treated by means of toxic drugs, serious consideration should be given to the danger of concomitantly enhancing the toxic effects of other necessary medications or stimulating already existing degenerative processes.

SUMMARY

Three deaths occurring in a series of elderly patients treated with urethane in chloroform water U.S.P. for carcinoma of the prostate gland are reported, and the results of the autopsies are briefly described. The possibility that the hepatic injury which played a role in at least two of these deaths was due to the synergistic action of urethane and chloroform was tested in rabbits. In these experiments the mortality and the survival data when considered along with histopathologic observations showed that urethane and chloroform are additively synergistic and that when given together, though in sublethal amounts, they can cause death from hepatic injury.

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RAPID DECELERATION AND RUPTURE OF THE AORTA

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THE theory of rapid deceleration[1] brought out to explain rupture of internal organs in aircraft accidents may be applied to explain rupture of the healthy aorta in certain types of automobile accidents when evidence of external violence is slight. Two cases illustrate this application:

CASE 1.—A man aged 56 was hurt in an automobile accident in which the car he was driving hit a concrete road block with sudden complete arrest of forward motion. About thirty minutes elapsed from the time of the accident until the patient was admitted to the hospital. The pulse was weak, rapid and thready. The patient was extremely pale and semiconscious. There were a few shallow lacerations over the sternum, the forehead and the rest of the face. There were no other marks on the body. The patient died five minutes after admission.

Autopsy showed a slight depression of the anterior wall of the chest on the left side. The third, fourth and fifth ribs were fractured in the midclavicular line. There was only slight displacement of the fragments, and the parietal pleura was not lacerated anteriorly. The left pleural cavity contained 4 liters of unclotted blood. The right contained 25 cc. of bloody fluid. The heart and the lungs showed no gross or microscopic lesions. The posterior mediastinum was infiltrated with about 1 liter of blood. When the aorta was exposed, a transverse rupture was observed at the junction of the arch and the descending aorta. The laceration was almost complete, with only 3 cm. of wall remaining intact on the medial aspect. The parietal pleura was ruptured at about the third dorsal vertebra, and this allowed blood to escape into the left pleural cavity.

Microscopic examination of sections of aorta at the site of rupture showed no medial disease and no arteriosclerosis of any significance.

CASE 2.—A man aged 34 was brought to the hospital with the information that he had been in an automobile accident. He was alone and details of the accident were not determined except that the car hit a tree. On admission the pulse was weak and thready. The respirations were shallow. The blood pressure could not be recorded. There was no external bleeding, and the only mark on the body was a shallow laceration around the right ear. This patient died fifteen minutes after admission.

Autopsy showed a shallow laceration of the right ear and a contusion of the right parietal region. No other marks or deformities were observed. Examination of the chest revealed a fracture of the left first rib, just lateral to the sternum.

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1. Hass, G. M.: J. Aviation Med. 15: 77, 1944.

The left pleural cavity contained 4 liters of unclotted blood. The heart and the lungs were normal grossly and microscopically. A transverse rupture of the aorta was found at the junction of the arch and the descending part. The laceration was almost complete; only 1 cm. of the wall remained intact. The left parietal pleura was ruptured at the fourth dorsal vertebra, apparently as a result of a marked increase of pressure in the posterior mediastinum; thus blood escaped into the left pleural cavity. There was about 50 cc. of bloody fluid in the abdominal cavity, and this appeared to come from a small laceration, 1 cm. in length, in the right lobe of the liver. Sections of various organs, including the aorta at the site of rupture, showed no pathologic changes.

COMMENT

It is easy to understand that in instances in which great violence is encountered actual tearing and rupture of vessels and organs may occur; but when there is minimal evidence of trauma, it is interesting to speculate on the mechanism of the rupture of the aorta. In cases reported in the literature by various authors[2] the rupture of the aorta occurred in certain locations, namely, just above the aortic valves or just below the attachment of the ductus arteriosus. Abbott[3] expressed the belief that there is a congenital weakness of the aorta in these regions. However, Rindfleisch[4] followed the idea that relative fixation of the aorta occurs where the great vessels are given off and that the degree of fixation varies according to the size of the vessels, so that distal to these locations the aorta is relatively mobile, and that it is the portions which are relatively fixed that are most prone to rupture.

Klotz and Simpson,[5] in attempting to cause rupture of the aorta by direct internal pressure, found that 1,000 mm. of mercury was not sufficient. Oppenheim,[6] by increasing the pressure to 3,000 mm. of mercury, did produce rupture, and it occurred primarily in either of the two aforementioned regions. This would tend to support the idea that the parts just above the valves and just below the attachment of the ductus arteriosus are weak points.

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2. (*a*) Busse, O.: Virchows Arch. f. Path. Anat. **183**:440, 1906. (*b*) Copeland, G. G.: J.A.M.A. **63**:1950, 1941. (*c*) Griffiths, S. J. H.: Brit. J. Surg. **18**:664, 1931. (*d*) Kuhn, L. P.: Illinois M. J. **47**:420, 1925. (*e*) Kemp, P. R.: Lancet **1**:953, 1923. (*f*) Jaffé, R. H., and Sternberg, H.: Vrtjschr. f. gerichtl. med. **58**:74, 1919. (*g*) Sherman, T. I.: J. Path. & Bact. **32**:795, 1929. (*h*) Collins, J. A., and D'Alessio, C. M.: New England J. Med. **219**:229, 1938, (*i*) Kleinsasser, L. J.: Ann. Surg. **118**:1071, 1943. (*j*) Narr, F. C., and Wells, A. H.: Am. Heart J. **8**:834, 1933. (*k*) Landau, J. V.: St. Barth. Hosp. J. **28**:63, 1931. (*l*) McDonald, J. B., and Campbell, W. A.: Am. Heart J. **30**:321, 1945.

3. Abbott, M. E.: Am. Heart. J. **3**: 392 and 574, 1928.

4. Rindfleisch, E.: Virchows Arch. f. path. Anat. **131**:374, 1873.

5. Klotz, O., and Simpson, W.: Am. J. M. Sc. **184**:455, 1932.

6. Oppenheim, F.: München. med. Wchnschr. **65**:1234, 1918; cited by Narr and Wells.[2j]

Sherman[2g] stated that since the ductus arteriosus joins the aorta near the root of the left lung and is closely related to the structures entering the root and since the whole mass there is supported by connective tissue which anchors it to the front of the vertebrae, the region of attachment of the ductus must act as if it were a hinge on which the aortic arch and the heart move. This must thus be a site at which, even in normal circumstances but particularly when the blood pressure is elevated, there is special strain.

None of the theories explains how the intravascular pressure is elevated to the point where rupture might occur, regardless of the nearly general agreement on the commonest sites.

The observations of Hass[1] on internal injuries of persons in aircraft accidents led him to conclude that "whenever one part of the body is decelerated at a rate which is different from that of another part of the body, the connection between these two parts is placed under stress which is proportional to the difference in the rates of deceleration." In cases of rapid deceleration the victim is momentarily subjected to the action of very large forces. These forces may be resolved along three principal axes, namely, vertical, horizontal or lateral. It seems to us that this theory most adequately explains the mechanism causing the elevation of intravascular pressure to the point that rupture of the aorta occurs. However, it fails to explain such cases as that reported by Kuhn[2d] in which a block of wood, or that by Kemp,[2e] in which a block of stone, struck the chest and caused rupture of the aorta, with signs of damage of the thoracic cage being minimal. In these instances the person involved was stationary, so he could not have been subjected to forces of deceleration.

In the cases now reported the rupture occurred in the commonest site, that is, just below the attachment of the ligamentum arteriosum arteriae pulmonalis, and in these cases, according to the theory of deceleration, the forces must have acted in the horizontal plane, thereby causing transverse laceration of all layers of the aorta and consequent fatal hemorrhage. In this respect these 2 cases are similar to cases reported by Hass.

SUMMARY

Two cases of rupture of a histologically normal aorta are reported in which the damage to the thoracic cage was minimal. Theories of such rupture are reviewed, and it would appear that in these cases it was most probably due to forces exerted by rapid deceleration. The theory of the effects of rapid deceleration which was brought out to explain ruptures of organs in military aircraft accidents, can be applied to rupture of organs in civilian automobile accidents in which there has been sudden arrest of forward motion. Therefore, in such cases, even in the absence of evidence of injury to the thoracic cage, the possibility of rupture of the aorta should be considered.

LESIONS PRODUCED IN CHICK EMBRYOS BY CANDIDA (MONILIA) ALBICANS

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MOORE[1] was the first to employ the chorioallantoic membrane of the embryonated egg as a medium for the study of lesions produced by pathogenic fungi (1939). The results of inoculating the membrane with a number of pathogenic fungi were reported by him in more detail in 1941[2]. Among the organisms studied was Candida (Monilia) albicans. These organisms when placed on the membranes of 12 day old chick embryos proliferated and formed large gray plaques on the ectoderm. Microscopically, the ectoderm was thickened and hyperkeratotic. Whorls of proliferating epithelial cells resembling "pearls" were observed in the mesoderm near the plaques. In the lesions both mycelial elements and yeastlike forms were associated with a pink-staining exudate composed largely of erythrocytes and monocytes. Fibroblasts were also proliferating. The embryos were all dead in six days. Similar granulomatous lesions due to Candida have been reported as observed in experimentally inoculated laboratory animals[3] and rarely as occurring in natural infection in man[4].

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This study was aided by a grant from the Special Research Fund of the University of Pennsylvania.

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2. Moore, M.: (*a*) Am. J. Path. **17**:103, 1941; (*b*) Am. J. Trop. Med. **21**:627 1941.

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Since Moore examined only the membranes of the embryos, it was our purpose to determine whether the tissues of the embryo will also respond to Candida infection by the formation of granulomas. Accordingly, suspensions of living and killed C. albicans, respectively, were inoculated in embryonated hens' eggs by three different routes; that is, they were applied to the chorioallantoic membrane, injected into the allantoic sac and injected into a vein of the chorioallantoic membrane.

MATERIALS AND METHODS

Embryonated hens' eggs previously incubated for eleven days were inoculated by the methods of Lee, Stavitsky and Lee[5] with suspensions of killed and living C. albicans. With suspensions of killed organism a concentration of 1:200 by volume was used throughout; but with suspensions of living organisms the concentration was varied in different experiments from 1:200 to 1:100,000. In one set of embryos the chorioallantoic membrane was inoculated with 0.1 cc. of organisms suspended in an isotonic solution of sodium chloride; in a second set the allantoic cavity was inoculated with 0.2 cc. of suspension, and in a third set 0.05 cc. of suspension was injected into one of the veins of the chorioallantoic membrane. By each of the three routes of inoculation a few embryos received an isotonic solution of sodium chloride alone. In addition a number of embryos were prepared for inoculation but were not inoculated. The last two groups served as controls. Following inoculation or preparation for inoculation, the eggs were incubated at 37 C. until individual embryos died or were killed, up to and including the time of hatching. Necropsies were performed on all embryos.

The strain of C. albicans employed was isolated from the sputum of a man. The organism was pathogenic for rabbits when injected either intratracheally or intravenously. The cultural and morphologic identification of the organism has been described.[6] The antigen for inoculation was prepared by culturing the organisms for eighteen to twenty-four hours on Sabouraud's glucose agar. The growth of organisms was then suspended in an isotonic solution of sodium chloride and adjusted to a concentration of 1:200 by volume. From this stock suspension further dilutions were made. When living organisms were injected, the

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Kurotchkin, T. J., and Lim, C. E.: Proc. Soc. Exper. Biol. & Med. **31**:332, 1933. (*k*) Ikeda, K.: Arch. Path. **22**:62, 1936; Am. J. Clin. Path. **7**:376, 1937; (*l*) Schattenberg, H. J., and Flinn, M.: Proc. Soc. Exper. Biol. & Med. **41**:557, 1939.

4. Boggs and Pincoffs.[3a] Warr, O. S.: Ann. Int. Med. **5**:307, 1931. Lewis, S. J.: Am. J. Clin. Path. **3**:367, 1933. Smith, L. W., and Sano, M. E.: J Infect. Dis. **53**:187, 1933. Flinn, J. W.; Flinn, R. S., and Flinn, Z. M.: Ann. Int. Med. **9**:42, 1935. Ikeda.[3k] Fiedman, N. B., and Donaldson, L.: Arch. Path. **27**:394, 1939. Joachim, H., and Polayes, S. H.: J.A.M.A. **115**:205, 1940. Koerth, C. J.; Donaldson, J. M., Jr, and McCorkle, R. G.: Am. Rev. Tuberc. **43**:723, 1941. Pasternack, J. G.: Am. J. Clin. Path. **12**:496, 1942. Wickler, A.; Williams, E. G.; Douglas, E. D.; Emmons, C. W., and Dunn, R. C.: J.A.M.A. **119**:333, 1942. Wickler, A.; Williams, E. G., and Wiesel, C.: Arch. Neurol. & Psychiat. **50**:661, 1943. Miale, J. B.: Arch. Path. **35**:427, 1943. Jaffin, A. E.: J. Mt. Sinai Hosp. **10**:586, 1944. Halpert, B., and Wilkins, H.: J.A.M.A. **130**:933, 1946.

5. Lee, H. F.; Stavitsky, A. B., and Lee, M.P.: Proc. Soc. Exper. Biol. & Med. **61**:143, 1946.

6. Rawson, A. J., and Norris, R. F.: Am. J. Clin. Path. **17**:807, 1947.

stock suspension and dilutions were prepared on the day of inoculation. When dead organisms were employed, a stock suspension was prepared at the beginning of the experiments by heating a 1:200 suspension of living organisms at 60 C. for one hour. This suspension was subcultured to assure sterility and was then kept in the refrigerator for further use.

Most embryos which died within twenty-four hours following inoculation were discarded. In the case of the remaining embryos the chorioallantoic membrane and the contents of the abdominal cavity were cultured. If contaminating organisms were present these specimens were also discarded. In the case of embryos inoculated with living Candida, the embryos were likewise discarded if Candida was not recovered on culture.

At necropsy the cranial and abdominal cavities were first sectioned. The chorioallantoic membranes and embryos were then placed in Regaud's solution. Blocks of chorioallantoic membrane, brain, neck, heart, lungs, liver, spleen, gizzard and kidneys were taken for section after fixation. Sections were stained routinely with hematoxylin and eosin. If lesions were present, duplicate sections were stained with Giemsa and with the gram stain of Brown and Brenn.[7]

RESULTS

The inoculation of 11 day old embryonated chicken eggs with killed C. albicans did not result in lesions of the chorioallantoic membrane or the embryo when the organisms were applied directly to the chorioallantoic membrane, were injected into the allantoic sac or were injected into one of the chorioallantoic veins. Likewise, when suspensions of living Candida in concentrations of 1:50,000 and 1:100,000 by volume were injected intravenously no definite lesions were observed in the chorioallantoic membrane or in the tissues of the embryo, although Candida was recovered in small numbers at necropsy. The application of living Candida in suspensions of 1:200 and 1:10,000 by volume to the chorioallantoic membrane and the injection of suspensions of 1:200 in the allantoic sac usually resulted in granulomatous lesions of the chorioallantoic membrane but under the conditions of the experiments did not result in granulomatous lesions of the embryo. In the case of those injected into the allantoic sac, however, there were focal and diffuse degenerative lesions in the embryos which were not certainly caused by Candida infection. Some of these may well have been postmortem changes. If the embryos survived more than twenty-four hours, intravenous injection of suspensions of living Candida in concentrations of 1:1,000 to 1:25,000 usually produced foci of necrosis or granulomas in the tissues of the embryo and in the chorioallantoic membrane. However, only a few embryos inoculated intravenously with large doses of Candida survived long enough for characteristic lesions to occur.

GENESIS OF LESIONS

After intravenous inoculation, the lesions caused by Candida in all of the organs were similar. In those embryos which died within twenty-four hours, focal lesions were not apparent. If Candida was seen, the organisms were scattered in large numbers in the tissues without inflammatory reaction. If the embryo survived for forty-eight hours, focal lesions were often present. These were characterized by circular or oval areas of liquefaction necrosis in which were large numbers of yeastlike and mycelial forms of Candida. Mycelial filaments of Candida

7. Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. **48**:69, 1931.

also extended about the periphery of the necrosis into the surrounding tissues. Although there were many erythrocytes in the necrotic areas, only a few inflammatory cells were present. These had slightly basophilic cytoplasm and a single nucleus, which was often indented. Small numbers of proliferating fibroblasts were also present (fig. 1). Many of the fixed tissue and parenchymal cells adjacent to the lesions and in contact with mycelial filaments showed marked karyorrhexis. This change was characterized by condensation of the nuclear chromatin into small markedly basophilic granules about the nuclear membrane. If the nuclear membrane was absent, the chromatin granules often gave the appearance of a necklace of beads. At times the granules were bunched together in small

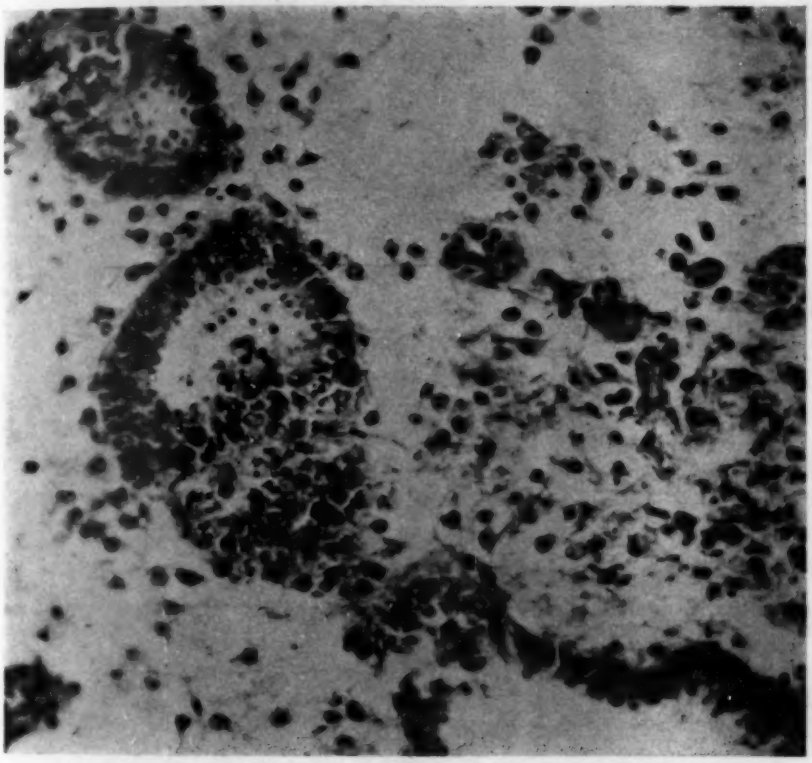

FIG. 1.—Lung of chick 113. Numerous mycelial filaments are present. Some of these are invading the bronchiole. At points of contact with the organisms, the epithelium is degenerating and shows marked karyorrhexis.

packets and both the nuclear and the cytoplasmic membranes are indistinct or absent (fig. 1).

In embryos which lived for three to four days after inoculation, large discrete abscesses were sometimes present. These lesions consisted of oval or circular areas of liquefaction necrosis in which were many yeastlike and mycelial elements of Candida, together with necrotic cells and pink-staining debris. Surrounding the

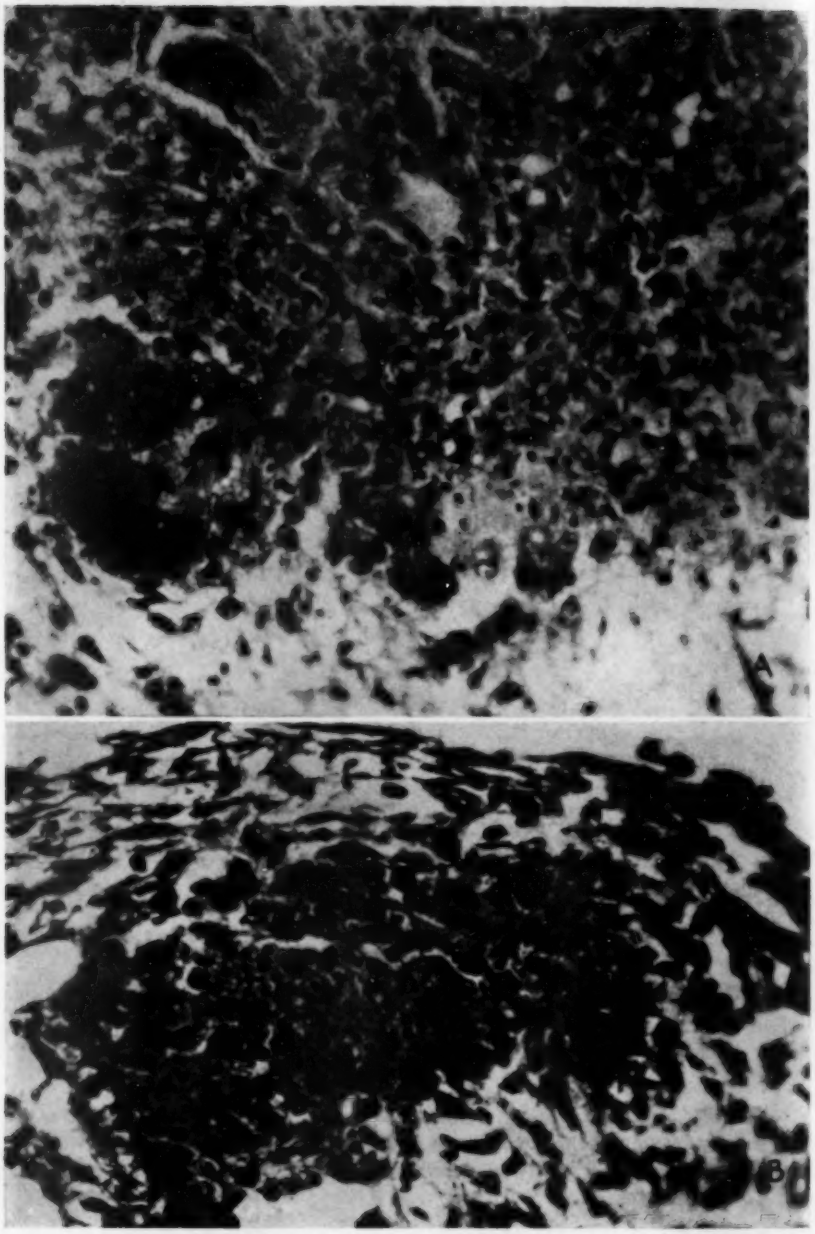

FIG. 2—*A*, lung of chick 179 four days after intravenous inoculation of a 1:25,000 suspension of living Candida. It shows the granulocytic exudate and the proliferation of fibroblasts and reticuloendothelial cells, many of which have coalesced to form giant cells.

B, liver of chick 173, showing a central area of densely packed inflammatory cells surrounded by a wide zone of proliferating fibroblasts.

areas of necrosis were zones of large mononuclear phagocytes or reticuloendothelial cells, many of which were coalescing to form multinucleated giant cells. In the cytoplasm of most of the giant cells phagocytosed Candida were visible. There were also small numbers of slightly acidophilic cells with indented segmented nuclei resembling polymorphonuclear leukocytes. There were few proliferating fibroblasts (fig. 2*A* and *B*). In some cases, however, the lesions were smaller and more numerous. Central areas of necrosis were small or lacking. Some were composed of densely packed masses of large mononuclear phagocytes and fibroblasts. The latter were also proliferating about the periphery of the lesions. Although many cells contained phagocytosed Candida, the organisms were much less numerous than in the larger abscesses (fig. 2*B*). In other areas the lesions were composed entirely of a central large multinucleated giant cell containing phagocytosed organisms and surrounded by mononuclear phagocytes and proliferating fibroblasts.

When lesions occurred on the chorioallantoic membrane from two to four days after inoculation, dome-shaped colonies of Candida were often growing on the surface as well as within the mesoderm of the membrane, whether the organisms had been applied directly to the membrane, injected into the allantoic sac or injected intravenously. In the case of the surface colonies, the cells of the outermost layer were predominantly yeastlike. The body of the colony was composed principally of mycelia at right angles to the surface, which extended into the underlying stroma of the membrane. At the point at which the colony was attached to the membrane, the surface epithelium was absent and was replaced by a zone of pink-staining necrotic exudate and organisms. Beneath this zone the mesoderm was infiltrated with polymorphonuclear leukocytes and by small numbers of oval cells with basophilic cytoplasm and eccentrically placed vesicular nuclei resembling plasma cells. Fibroblasts were also proliferating, and there were scattered collections of large mononuclear phagocytes and multinucleated giant cells containing ingested Candida. In addition, usually lateral to the colonies, in localities in which the surface epithelium was not denuded, there were peglike processes of squamous epithelial cells extending into the mesoderm. These projections were composed of central cores of keratotic epithelium, about which were cells resembling the basal layer of ectoderm. Mitotic figures were seen in only the outer layers of epithelium.

COMMENT

The granulomas which resulted in the tissues of the embryo after intravenous inoculation of living C. albicans were similar to the lesions of the chorioallantoic membrane previously reported by Moore[2a]. The character of the lesions also resembled those described in man[4] and in experimentally inoculated animals[3].

In the preceding description we made no mention of the presence of lymphocytes in the inflammatory exudate of the lesions. In sections stained with hematoxylin and eosin, lymphocytes were seldom recognized in either blood vessels or lesions. With Giemsa stains, however, they were often identified in blood vessels but not in the lesions. Moore[2a] likewise did not mention the presence of lymphocytes in the lesions of the chorioallantoic membrane, nor did Canat and Opie[8]

8. Canat, E. H., and Opie, E. L: Am. J. Path. **19**:371, 1943.

discuss lymphocytes in their study of inflammation in the chick embryo. However, Sabin[9] found that lymphocytes become abundant in the blood stream of the chick embryo at about the fifth or sixth day of incubation. From the results of the present experiments, therefore, it is uncertain whether the lymphocytes present in the blood stream play an active part in the inflammatory reaction of Candida.

The hyperkeratosis of the ectoderm with the formation of cylindric projections of epithelium in the mesoderm described by Moore[2a] and ourselves as a feature of the response of the chorioallantoic membrane to infection with Candida is not a specific or a unique lesion. In 1918 Danchakoff[10] described identical metaplastic changes of the ectoderm occurring when grafts of adult spleen were implanted on the chorioallantoic membrane.

SUMMARY

The lesions caused by C. albicans in the tissues of the embryo when inoculated intravenously in 11 day old embryonated hens' eggs were similar to those produced in the chorioallantoic membrane. The lesions also resembled those reported in man and in animals experimentally inoculated with C. albicans.

If the chick embryo is not quickly overwhelmed by the infection, there are at first focal areas of liquefaction necrosis, associated with hemorrhage and scanty granulocytic infiltration. As the lesions become older, the granulocytic infiltration becomes greater and larger numbers of mononuclear phagocytes and multinucleated giant cells, accompanied by proliferating fibroblasts, surround the focal necroses.

9. Sabin, F. R.: Bull. Johns Hopkins Hosp. **32**:314, 1921.
10. Danchakoff, V.: Am. J. Anat. **24**:127, 1918.

EXTRA-ABDOMINAL DESMOID TUMORS

Their Differential Diagnosis and Treatment

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BY THE TERM "desmoid tumor" one generally infers a hard, fibrous tumor occurring in the abdominal parietes. However, the term has also been employed to indicate similar tumors occurring elsewhere than in the abdominal wall. The present study is based on a careful clinicopathologic examination of 34 extra-abdominal tumors which were removed surgically at the Mayo Clinic in the years 1908 to 1945 inclusive. In addition, an equal number of tumors diagnosed as low grade fibrosarcoma were taken at random from the clinic files and studied microscopically, in a similar manner, in an attempt to draw up differential histologic and cytologic criteria between the desmoid tumor and fibrosarcoma. As most of the literature concerning desmoid tumors has been written about those arising in the abdominal wall, reference will, of necessity, be frequently made to these articles.

DEFINITION

Desmoid tumors are benign fibrous neoplasms which arise from the musculoaponeurotic structures throughout the body and which have the peculiar characteristic of locally invading adjacent muscle tissues and engulfing the striped muscle fibers. Therefore, morphologically they have the local cancerous property of invasion, but histologically they are noncancerous.

HISTORICAL SUMMARY

In 1832 Marfarlane,[1] of Glasgow, Scotland, accurately described 2 tumors occurring "between the layers of the abdominal muscles," which were almost certainly desmoid tumors. The term "desmoid"

From the Section on Surgical Pathology, Mayo Clinic.

1. Macfarlane, J.: Clinical Reports of the Surgical Practice of the Glasgow Royal Infirmary, Glasgow, D. Robertson, 1832, pp. 63 and 66.

(δεσμός, a band or tendon; εἶδος, appearance) was coined by Müller[2] in 1838 to designate tumors of a tendon-like consistency.

The first description of the microscopic appearance of these tumors was given by Bennett[3], in 1849. He described and depicted, by means of woodcuts, the microscopic characteristics of 3 growths occurring on the thigh, in the parotid region and on the arm, respectively. "These tumors all recurred after local excision but none of these metastasized." In 1856 Paget[4] reported 2 cases of desmoid tumor; in one the lesion occurred in the abdominal wall, and in the other, on the forearm. "Under the microscope, the tumor of the forearm was found to be composed of two materials—nuclei, and a sparingly granular or molecular substance in which they were imbedded. These characters were found to be present in the tumor (of the abdominal wall) from this man, thus showing their identity with each other." Thus, the first description of the microscopic appearance of a desmoid tumor was that of an extra-abdominal growth, and Paget recognized that the abdominal and the extra-abdominal neoplasm are one and the same.

In the years 1881 to 1885 Sänger[5] published four papers on desmoid tumors of the abdominal wall so that, in this manner, the term "desmoid" became linked with a specific entity. This designation has continued to be used by most German and English-speaking writers, although the term is confusing, for some authors continue to use it to indicate both "benign" and "malignant" tumors. Another confusing point is that Pfeiffer[6], in 1904, limited the term "desmoid" to "fibromas arising in the musculo-aponeurotic structures of the abdominal walls," and this has been the generally held conception since that time. The French and a few of the English-speaking writers still refer to this tumor as a "fibroma." It is also known as a "desmoma" by the English.

Nichols,[7] in 1923, was the first author to call the extra-abdominal fibrous tumors "desmoids." He reported 6 cases of extra-abdominal desmoid tumor along with 25 cases of desmoid tumor of the abdominal wall and stressed the similarity in the gross and the microscopic appearance of these tumors, regardless of their situation. The following authors have also reported cases of fibroma, desmoma or desmoid tumor in which the lesion occurred in the musculoaponeurotic struc-

2. Müller, J.: Ueber den feinern Bau und die Formen der krankhaften Geschwülste, Berlin, G. Reimer, 1838.

3. Bennett, J. H.: Lancet **2**:428, 1849.

4. Paget, J.: Lancet **1**:625, 1856.

5. Sänger, M.: Arch. f. Gynäk. **24**:1, 1884.

6. Pfeiffer, C.: Beitr. z. klin. Chir. **44**:334, 1904.

7. Nichols, R. W.: Arch. Surg. **7**:227, 1923.

tures elsewhere than the abdominal wall: Guyon[8], Auvray[9], Bellanger[10], Marlow[11], Hoffmeister[12], Woytek[13], Cigolini[14], Salto[15], Mason[16], Waugh[17], Pearman and Mayo[18], Wiper and Miller[19].

CAUSATION

Numerous theories have been advanced in attempts to explain the origin of desmoid tumors. However, there are only two theories tenable today, the first being that the tumor is related to trauma and the second being that it is based on an endocrinologic disturbance.

1. In each of the 2 cases reported by Paget[4] in 1856 the lesion had been preceded by a definite injury, a wound of the forearm in the first and a blow on the abdominal wall in the second. In 1880 Ebner[20] advanced the theory that "desmoids" resulted from muscular rupture, due either to violent muscular contraction or to trauma from external sources. In Stewart and Mouat's[21] series of 63 abdominal desmoid tumors, 9 were preceded by a "blow or other injury to the affected part." Labbé and Remy[22] were the first to put forward the concept that the great preponderance of abdominal desmoid tumors in women as compared with men (ratio of 7:1) was due to the violent muscle contractions of labor. Waugh[17] reported an abdominal desmoid tumor in a young boy which followed an injury to the abdominal wall, and stressed the possibility of trauma as an etiologic basis for such tumors. A well known antecedent factor is the relationship between an abdominal incision and subsequent desmoid formations. Several authors[23] have reported neoplasms of this type in postoperative scars. Thus, to sum up the first theory, trauma of three types seems to play a part: (1) external trauma, (2) the physiologic trauma of labor and (3) the trauma of a previous surgical incision. In

8. Guyon, F.: Tribune méd. **10**:257, 277, 309 and 354, 1877; abstracted, Bull. Acad. de méd. **6**:546, 1877.

9. Auvray, M.: Bull. et mém. Soc. nat. de chir. **56**:103, 1930; **60**:1236, 1934.

10. Bellanger, M. H.: Bull. et mém. Soc. de chir. de Paris **27**:366, 1935.

11. Marlow, F. W.: Canad. M. A. J. **32**:674, 1935.

12. Hoffmeister, W.: Zentralbl. f. Chir. **62**:1888, 1935.

13. Woytek, G.: Zentralbl. f. Chir. **57**:770, 1930.

14. Cigolini, G.: Boll. e mem. Soc. piemontese di chir. **5**:1002, 1935.

15. Salto, G.: Osp. maggiore, **23**:485, 1935.

16. Mason, J. B.: Ann. Surg. **92**:444, 1930.

17. Waugh, J. M.: Am. J. Surg. **50**:694, 1940.

18. Pearman, R. O., and Mayo, C. W.: Ann. Surg. **115**:114, 142.

19. Wiper, T. B., and Miller, J. M.: Am. J. Surg. **71**:556, 1946.

20. Ebner, L.: Berl. klin. Wchnschr. **17**:528, 1880.

21. Stewart, M. J., and Mouat, T. B.: Brit. J. Surg. **12**:355, 1924.

22. Labbé L., and Remy, C.: Traité des fibromes de la paroi abdominale, Paris, A. Delahaye & E. Lecrosnier, 1888.

14 of 34 cases of extra-abdominal desmoid tumor of the present investigation, the patient definitely linked the onset of the tumor with trauma. One of the tumors developed postoperatively in the scar of a radical mastectomy.

2. The endocrine theory of the origin of the desmoid tumor was suggested by Geschickter and Lewis[23f] in 1935. They did bioassays of estrogen and the anterior pituitary gonadotropic substance on several connective tissue tumors, one of which was a desmoid tumor of the abdominal wall. The latter tumor contained 13,000 rat units of gonadotropic substance per kilogram of tissue, but no estrogen. A keloid, likewise examined, contained both estrogen and gonadotropic substance. These authors concluded that these endocrine substances may play a part in the causation of connective tissue tumors and that they are probably "concerned in the physiology of the tumors."

Lipschütz and co-workers[24] have done a great deal of experimental work on the formation and the prevention of connective tissue tumors in guinea pigs. With estrogenic substances they have been able to produce fibromyoma in the uterus and in different abdominal organs or in the abdominal wall in 100 per cent of cases. Tumors of this type, which consist mainly of fibroblasts, are capable of invading and destroying smooth and striated muscle as well as other tissues. However, they are "benign" tumors, incapable of metastasizing, and when the administration of estrogen is stopped, they regress in size. Male guinea pigs are not immune to these growths. These authors have shown conclusively that testosterone, progesterone and desoxycorticosterone can prevent the formation of these experimentally produced fibromyomas in guinea pigs.

Pack and Ehrlich[25] discussed a hormonal hypothesis for the development of desmoid tumors in man and reported a case in which an inoperable desmoid tumor of the abdominal wall occurring in a 28 year old woman was treated by "radiation castration." This tumor involved the right lower abdominal quadrant, the ilium and the retroperitoneal tissue. An attempt at operative removal in 1936 was

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23. (*a*) Andrews, E. W.: Surg., Gynec. & Obst. **12**:190, 1911. (*b*) Balfour, D. C.: Railway Surgeon's J. **22**:434, 1916. (*c*) Bessesen, D. H.: Am. J. Surg. **16**.513, 1932. (*d*) Danforth, W. C.: Surg., Gynec. & Obst. **29**:175, 1919. (*e*) Esau: Arch. f. klin. Chir. **164**:713, 1931. (*f*) Geschickter, C. F., and Lewis, D.: Am. J. Cancer, **25**:630, 1935. (*g*) Penick, R. M.: Internat. S. Digest, **23**:323, 1937. (*h*) Walters, W., and Church, G. T.: S. Clin. North America, **14**:647, 1934. (*i*) Pearman and Mayo.[18] (*j*) Stewart and Mouat.[21]

24. Lipschütz, A., and Grismali, J.: Cancer Research **4**:186, 1944. Lipschütz, A., and Vargas, L.: Lancet **1**:1313, 1939. Lipschütz, A., and Zañartu, J.: Endocrinology **31**:192, 1942. Vargas, J., Jr.: Bull. Johns Hopkins Hosp. **73**:23, 1943.

25. Pack, G. T., and Ehrlich, H. E: Internat. Abstr. Surg. **79**:177, 1944.

unsuccessful. A course of roentgen therapy was given to the tumor site at that time. This was repeated later in the year. "The neoplasm continued to grow and the menses were unimpaired." In 1938 sufficient pelvic irradiation was given to bring on an artificial menopause, and the neoplasm began to regress. When last examined in 1943, the mass was much smaller and "completely quiescent."

To date we have been unable to find a report of a case of a desmoid tumor treated with one of the previously mentioned hormones which were shown by Lipschütz and co-workers[24] to be antifibromatogenic.

In addition to the etiologic factors of trauma and hormonal stimulation there must be an unknown fibromatogenic principle to explain the formation of these tumors. This principle or factor is amply exemplified in Straub's[26] report of the formation of keloids in 3 of 4 postoperative scars which followed removal of desmoid tumors of the abdominal wall. Another example of this fibromatosis is found in Marlow's[11] case in which he removed a desmoid tumor from the trapezius muscle of a 26 year old woman and in which, eight years later, he removed a fibroadenoma from the breast.

INCIDENCE, AGE AND SEX OF PATIENTS

Since most of the literature concerning desmoid tumors has been written about the neoplasms situated in the anterior abdominal wall, one must refer to these articles to gain an idea of the frequency of this tumor. Billroth[27] saw 16 abdominal "desmoids" in twenty-three years; Stone[28] mentioned 5 encountered in 21,000 surgical patients at Johns Hopkins Hospital, and Pack and Ehrlich[25], in the years 1917 to 1943, inclusive, observed 17 desmoid tumors of the abdominal wall among 50,346 cases of neoplastic disease, an incidence of 0.03 per cent.

It is much more difficult to depict accurately the incidence of the desmoid tumors that occur elsewhere than in the abdominal wall, owing to the varying names and definitions of this neoplasm. The authors listed in the historical summary[29] have described 15 tumors which fall into the extra-abdominal desmoid class as defined earlier in this article. Listed in the files of the Mayo Clinic for the years 1906 to 1945, inclusive, are 45 extra-abdominal desmoid tumors and 85 desmoid tumors of the abdominal wall.

Eleven of the 45 cases of extra-abdominal desmoid tumors had to be discarded from the present study for the following reasons: (1) in

26. Straub, G. F.: California & West Med. **31**:186, 1929.

27. Billroth, cited by Labbé and Remy,[22] p. 75.

28. Stone, H. B.: Ann. Surg. **48**:175, 1908.

29. Bennett.[3] Paget.[4] Guyon.[8] Auvray.[9] Bellanger.[10] Marlow.[11] Hoffmeister.[12] Woytek.[13] Cigolini.[14] Salto.[15] Wiper and Miller.[19]

2 the tumor had been lost, (2) the specimen was unsatisfactory in 7 cases and (3) in 2 cases the patient came to the clinic with microscopic sections of a tumor that had been removed elsewhere. The present investigation is therefore based on an examination of 34 cases of extra-abdominal desmoid tumor. The patients ranged in age from 9 to 71 years, with an average age of 40. There were 10 males and 24 (70 per cent) females.

SYMPTOMS

There is essentially one sign and one symptom of a desmoid tumor, namely, a lump and pain. The usual history is that the patient feels a lump and shortly thereafter begins to associate aches and pains with the lump. Occasionally, a major nerve may be caught in the advancing tumor, the pain then being severe; on the other hand, when a nerve is not involved, the pain is usually mild and aching in character.

MORBID ANATOMY

The definition of desmoid tumors given in these pages quite accurately depicts the gross characteristics of these neoplasms. What has been written in the literature regarding tumors of the abdominal wall could be repeated almost verbatim for the extra-abdominal desmoid tumors.

To date, all the extra-abdominal desmoid tumors reported have been solitary. All 34 of the present series were single. The size varied from 1 to 15 cm. in diameter, with an average of 6 cm. The tumors tended to be oblong, with the long axis lying in the direction of the muscle fibers, that is, in the direction of least resistance. None of the tumors was truly encapsulated; each was invariably fixed to the surrounding musculoaponeurotic or osseous structures or both at one or more points. However, a few of the tumors could be partially enucleated with the finger. This apparent encapsulation occurred at the site at which the tumor was compressed against an aponeurosis, an intermuscular septum or bone. Figure 1 shows the location of the 34 extra-abdominal desmoid tumors.

Desmoid tumors have a hard, rubbery consistency and give a creaking sensation when cut with the knife. The cut surface of the small and medium-sized tumors is a glistening, whitish pink color, with numerous interlacing bundles of white fibrous tissue, much like fibroids of the uterus. The larger tumors tend to show areas of myxomatous and cystic degeneration due to lack of central blood supply. The most interesting feature of the cut surface is the inclusion of bundles of muscle fibers in the periphery of the tumor.

The muscle fibers give the impression of penetrating the tumor for an average of 2 to 4 mm. The aforementioned gross characteristics of these extra-abdominal desmoid tumors are well shown in figure 2.

HISTOLOGIC ASPECTS

Desmoid tumors present a microscopic picture varying from that of an acellular to that of a fairly cellular fibroma plus incorporated striated muscle fibers. Stewart and Mouat[21] gave an excellent descrip-

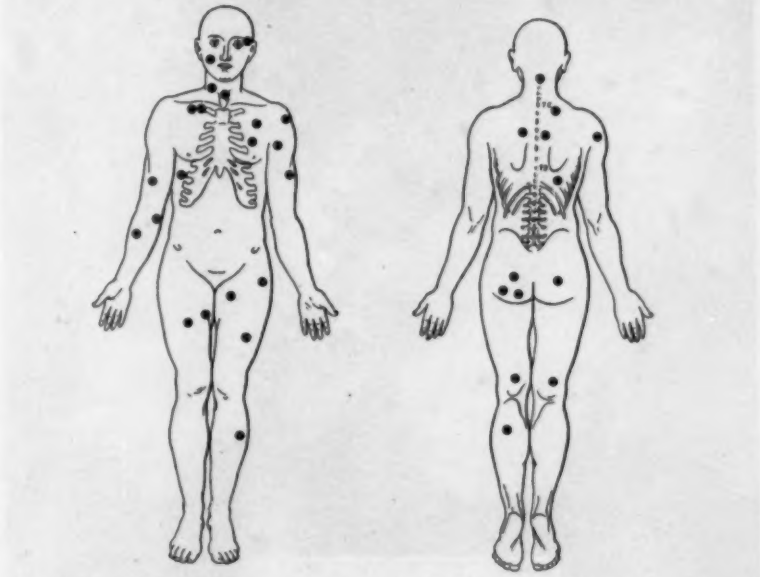

Fig. 1.—Location of 34 extra-abdominal desmoid tumors.

tion of these muscle inclusions. They described three variations in the muscle cells as these are engulfed in the tumor: The cells at the periphery have the normal appearance of striated muscle; the intermediary cells lose their striations and become like smooth muscle cells in appearance, and the most deeply placed are multinucleated, giant-cell-like protoplasmic masses. They described this metamorphosis of the muscle fibers as being due to loss of function and nerve supply, resulting in reversion to primitive muscle cells. The more widely accepted theory of this change is that the engulfed muscle fibers become strangled by the advancing fibroblasts of the neoplasm so that the muscle cells undergo progressive degeneration[25].

Method of Examination.—In the present study the microscopic examination was carried out in a definite manner. Blocks of tissue were cut from the edge and the center, respectively, of each of the specimens which had been fixed in formaldehyde

solution, U.S.P. The blocks were then placed in paraffin, and uniform sections, 8 microns thick, were cut and stained with hematoxylin and eosin. The size of the stained sections averaged 1 sq. cm. These sections were then examined before recourse to the histories was taken, in order to prevent the knowledge of clinical outcome from influencing the results of microscopic examination.

Each section of tissue was examined in its entirety by means of a mechanical stage. The histologic examination was carried out under low power (× 100) mag-

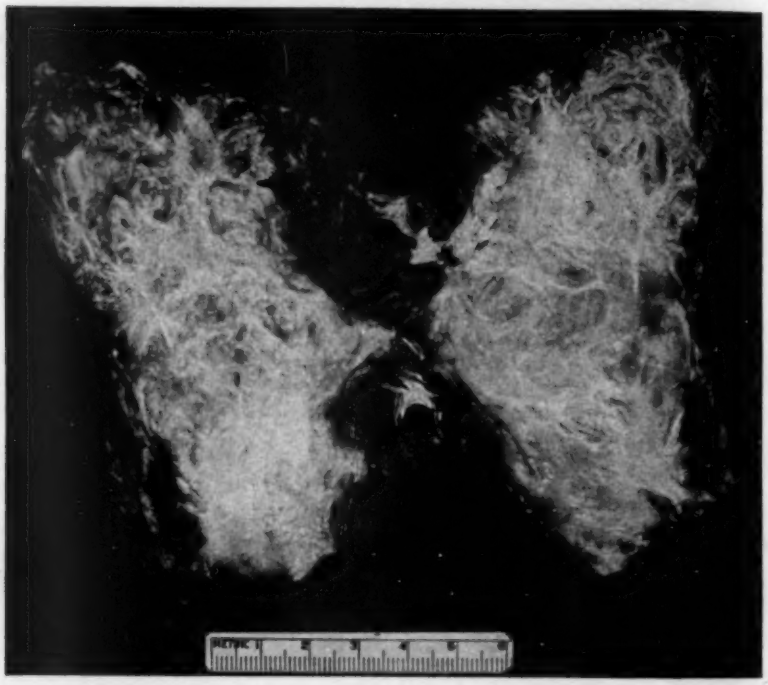

Fig. 2.—Cut surface of an extra-abdominal desmoid tumor, showing the interlacing bundles of white fibrous tissue and the engulfed muscle fibers around the periphery.

nification, with corroboration, when necessary, under high magnification (× 450), with the dry lens, and the cytologic examination was done entirely under high magnification with the dry lens. The microscopic features specifically noted are shown in table 1. All the neoplasms showed muscle inclusions. The actual number of mitotic figures, of pathologic mitotic figures and of tumor giant cells was counted, whereas the remainder of the characteristics were graded on a basis of 1 to 4 in which 1 represented the minimal and 4 the maximal condition.

COMMENT ON THE MICROSCOPIC APPEARANCES

Muscle Inclusion: A desmoid tumor must contain engulfed striated muscle fibers to fulfil the characteristics noted in the definition of this neoplasm (figs. 2 and 3 *A* and *B*). If the section to be

TABLE 1.—*Microscopic Characteristics of Thirty-Four Extra-Abdominal Desmoid Tumors*

											Cellular Variation†			
Case	Muscle Inclusion*	Center Older Than Periphery	Myxomatous Degeneration†	Hemosiderin†	Inflammatory Cells†	Encapsulation†	Cellularity†	Mitotic Figures‡	Pathologic Mitotic Figures‡	Tumor Giant Cells‡	Size	Shape	Staining	Nucleoli
1	+	0	0	0	0	0	2	7	0	0	1	1	1	1
2	+	0	0	0	0	0	2	1	0	0	0	0	0	0
3	+	0	1	0	2	0	2	0	0	0	1	1	0	0
4	+	0	0	0	0	0	2	0	0	0	0	0	0	0
5	+	0	0	0	1	0	2	0	0	0	0	0	0	0
6	+	0	0	0	2	0	1	0	0	0	0	0	0	0
7	+	0	0	0	1	1	3	1	0	0	1	1	2	2
8	+	1	0	0	0	0	3	2	0	0	1	1	0	0
9	+	0	0	0	1	0	2	0	0	0	0	0	0	0
10	+	0	3	0	0	0	1	0	0	0	0	0	0	0
11	+	0	0	0	0	0	2	0	0	0	0	0	0	0
12	+	0	0	0	1	0	2	0	0	0	0	0	0	0
13	+	0	2	0	1	1	2	0	0	0	0	0	0	0
14	+	0	0	0	1	0	1	0	0	0	1	1	1	1
15	+	0	0	0	1	0	3	2	0	0	2	2	1	2
16	+	0	0	0	1	0	1	0	0	0	0	0	0	0
17	+	0	0	0	1	0	2	0	0	0	0	0	0	0
18	+	0	0	0	0	0	1	0	0	0	0	0	0	0
19	+	0	0	0	0	0	2	0	0	0	0	0	0	0
20	+	0	0	0	1	0	2	2	0	0	1	1	1	1
21	+	0	4	0	2	0	1	0	0	0	0	0	0	0
22	+	0	0	0	0	0	2	1	0	0	0	0	0	0
23	+	0	0	0	2	0	2	2	0	0	1	1	1	1
24	+	0	1	0	2	0	2	0	0	0	0	0	0	0
25	+	0	0	0	0	0	1	0	0	0	0	0	0	0
26	+	1	0	1	2	0	2	2	0	0	1	1	0	0
27	+	0	0	0	2	1	3	2	0	0	2	2	1	2
28	+	0	0	0	0	0	2	0	0	0	0	0	0	0
29	+	0	0	1	1	0	2	3	0	0	2	2	1	2
30	+	0	0	0	1	0	2	0	0	0	0	0	0	0
31	+	0	0	0	0	0	2	0	0	0	0	0	0	0
32	+	0	0	0	0	0	1	0	0	0	0	0	0	0
33	+	0	3	1	0	0	2	0	0	0	0	0	0	0
34	+	0	0	0	1	0	2	0	0	0	0	0	0	0

*The presence of this characteristic is indicated by +; its absence, by 0.
†This characteristic is graded on a basis of 1 to 4, in which 1 represents the minimal and 4 the maximal condition; 0 means absence of the indicated characteristic.
‡The number per section is recorded.

examined is cut from that portion of the tumor which is compressed against an aponeurosis or periosteum, there will, of course, be no muscle inclusion. However, if the section is cut from a portion of the lesion which involves the surrounding muscle, the engulfed muscle

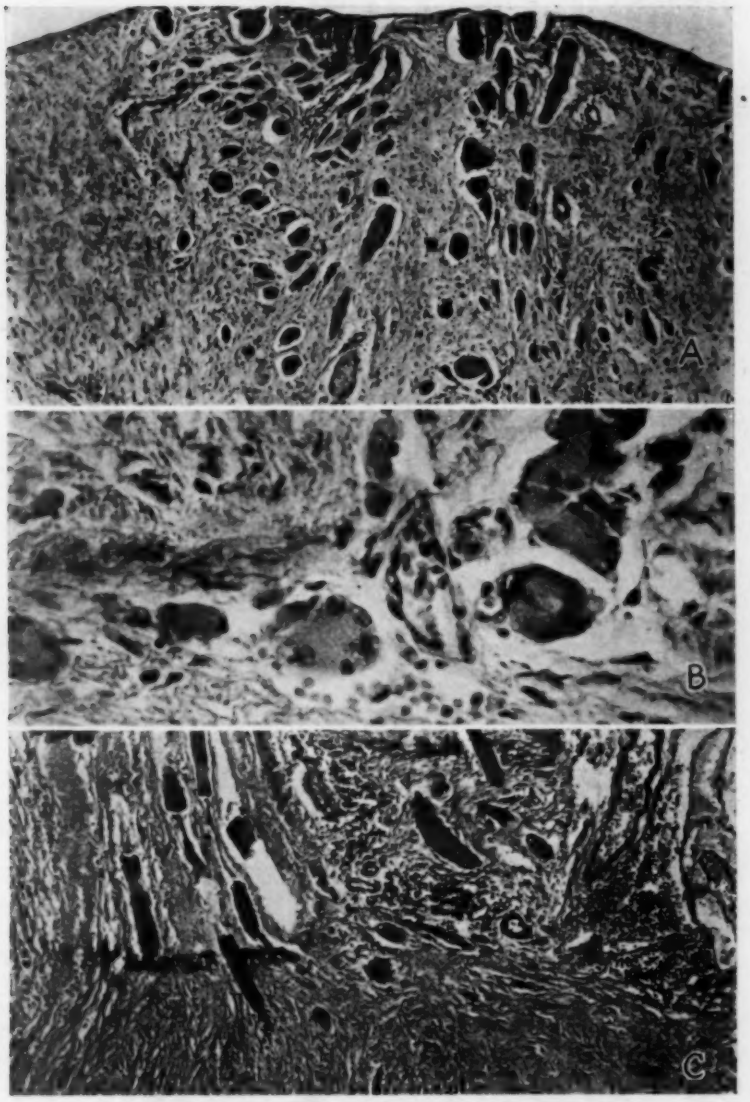

Fig. 3.—*A*, engulfed striated muscle cells in the edge of a desmoid tumor (hematoxylin and eosin; × 65).

B, muscle giant cells (hematoxylin and eosin; × 415).

C, chronic inflammatory cells surrounding muscle fibers in the edge of a desmoid tumor (hematoxylin and eosin; × 90).

fibers will be readily found. Occasionally, the coarse collagen bundles of a fascial sheath or an intermuscular septum will be mistaken microscopically for muscle fibers which have lost their striations. This difficulty is readily settled by using Van Gieson's stain, which causes the collagen bundles to show up bright red and the muscle fibers yellow.

Center Older Than Periphery of Tumor: Most of the descriptions of the microscopic appearance of this neoplasm state that the growing edge is younger and more active than the central portion. This observation could be corroborated in only 2 of the 34 cases included in the present study.

Myxomatous Degeneration: This type of degeneration is not uncommon in connective tissue tumors. A good example of such degeneration is that of the mucoid material seen in the center of a large uterine fibroid. This change is usually brought about by loss of a central blood supply, but one should always be suspicious of an appearance of myxosarcoma and should carry out a thorough microscopic examination.

Hemosiderin: Every section was carefully examined for hemosiderin, for a specific reason. As noted previously, the most widely accepted theory of the causation of desmoid tumors is that they are related to trauma. The muscle is ruptured either by a powerful contraction or by a direct blow, with the formation of a hematoma, which undergoes organization. The fibroblasts of this organized mass continue to proliferate beyond the stage of repair, so that a fibrous neoplasm is formed. If this theory is correct, one would expect to find hemosiderin crystals, the leftovers from the previous hematoma, scattered throughout the new growth. In only three sections were traces of hemosiderin found. From this one could draw two conclusions: first, that the hemosiderin crystals had been entirely phagocytosed or, second, that in the majority of cases the neoplasm had not originated on the basis of trauma and formation of a hematoma.

Inflammatory Cells: Twenty-one of the extra-abdominal desmoid tumors showed lymphocytic infiltration in the region of the muscle inclusions around the periphery (fig. 3*C*). To be certain that this finding was not limited to the extra-abdominal desmoid tumors, 20 desmoid tumors of the abdominal wall were examined. Sixteen of the 20 tumors showed similar lymphocytic involvement. Boyd[30] stated that this lymphocytic infiltration is often found in the advancing edge of a neoplasm. There is no indication that the desmoid tumors had been formed as a result of the inflammation.

30. Boyd, W.: Surgical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1942.

The remaining microscopic features shown in table 1, namely, the histologic changes of encapsulation and cellularity and the cytologic characteristics of mitosis, pathologic mitosis, tumor giant cells and variation in cellular size, shape, staining and nucleoli, were specifically noted in an attempt to formulate criteria which would aid in making the microscopic differentiation between the desmoid tumor and fibrosarcoma. Therefore, these findings will be discussed further in conjunction with the study of fibrosarcoma.

MICROSCOPIC STUDY OF FIBROSARCOMA

Table 2 depicts the microscopic observations in 34 tumors which had been diagnosed as low grade (grades 1 and 2) fibrosarcoma. By comparison of the characteristics of the desmoid tumors shown in table 1 with those of the specimens of fibrosarcoma given in table 2, the following interesting variations between the two types of tumors were noted: the fibrosarcoma was more often encapsulated; it showed a higher degree of cellularity, more mitotic figures (fig. 4*A*), a considerably greater variation in the size, shape, staining and nucleoli of the fibrosarcomatous cells, and, in addition, numerous pathologic mitotic figures and tumor giant cells.

COMPARATIVE MICROSCOPIC STUDY OF DESMOID TUMORS AND FIBROSARCOMAS

Encapsulation.—By encapsulation one infers an abutment of pathologic cells against a surrounding buttress of normal cells. This is well shown in figure 4B. As more of the neoplastic cells grow out and penetrate between the cells of the surrounding tissues, the encapsulation becomes less marked. Figure 3*A* shows a desmoid tumor without any attempt at encapsulation. Thirty-one of the 34 desmoid tumors showed no encapsulation, the remaining 3 being only slightly encapsulated (table 1), whereas 30 of the 34 specimens of fibrosarcoma were encapsulated (table 2). These microscopic features fit in well with the gross characteristics of these two tumor types, for the desmoid tumors grow into the surrounding muscle and engulf the muscle fibers, whereas the tumors diagnosed as fibrosarcoma "are rounded or lobulated and more than half are encapsulated. Those not encapsulated are usually exceedingly well circumscribed" (Broders, Hargrave and Meyerding[31]). Therefore, encapsulation is an important point in both the gross specimen and the microscopic section, in the differentiation between the desmoid tumor and fibrosarcoma.

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31. Broders, A. C.; Hargrave, R., and Meyerding, H. W.: Surg., Gynec. & Obst. **69**:267, 1939.

TABLE 2.—*Microscopic Characteristics of Thirty-Four Tumors Diagnosed as Low Grade Fibrosarcoma*

Case	Muscle Inclusion*	Myxomatous Degeneration†	Inflammatory Cells†	Encapsulation†	Cellularity†	Number‡			Cellular Variation†			
						Mitotic Figures‡	Pathologic Mitotic Figures‡	Tumor Giant Cells‡	Size	Shape	Staining	Nucleoli
35	+	0	1	1	3	135	4	19	3	3	3	3
36	0	0	1	4	3	8	0	0	0	0	0	0
37	+	1	2	1	3	41	3	49	2	2	3	2
38	+	1	0	2	3	3	0	8	2	2	3	1
39	0	0	0	1	4	3	0	0	1	1	1	0
40	0	1	1	2	3	2	0	0	1	1	1	0
41	0	3	2	3	2	4	0	2	1	1	1	1
42	0	1	0	2	2	3	0	0	1	1	1	1
43	0	1	0	4	2	3	0	2	0	0	0	0
44	0	0	1	3	3	30	0	0	2	2	2	1
45	0	0	1	4	3	5	0	0	2	1	2	2
46	0	1	2	2	4	8	0	0	1	1	1	1
47	+	1	1	2	3	60	1	0	3	2	2	3
48	+	0	1	1	3	30	0	9	3	2	2	2
49	0	2	0	0	4	12	0	5	2	2	3	2
50	0	0	0	0	4	46	0	0	1	1	1	1
51	0	0	0	0	4	1	0	0	0	0	0	0
52	+	1	2	1	3	40	2	27	3	2	2	2
53	0	0	0	2	4	17	0	0	0	0	0	0
54	0	1	1	1	3	13	0	3	3	2	2	1
55	0	1	0	2	3	11	0	32	2	2	2	1
56	0	0	2	3	3	76	5	105	4	3	3	4
57	0	0	0	2	2	59	0	2	2	2	2	2
58	+	1	1	2	2	41	2	40	2	1	2	2
59	+	1	1	2	3	16	1	9	1	1	2	2
60	0	0	1	1	3	9	5	36	3	3	2	3
61	0	0	1	4	3	33	1	28	1	1	1	1
62	+	2	1	1	2	18	1	21	2	2	3	2
63	0	0	0	3	4	12	0	0	1	1	1	1
64	0	0	0	2	3	51	11	175	3	3	3	3
65	+	0	2	0	3	30	2	16	2	2	2	2
66	+	2	1	2	2	8	0	2	2	2	1	1
67	+	0	0	2	3	80	15	50	2	2	2	3
68	0	0	0	3	4	2	0	2	1	1	1	1

*The presence of this characteristic is indicated by +; its absence, by 0.

†This characteristic is graded on a basis of 1 to 4, in which 1 represents the minimal and 4 the maximal condition; 0 means absence of the indicated characteristic.

‡The number per section is recorded.

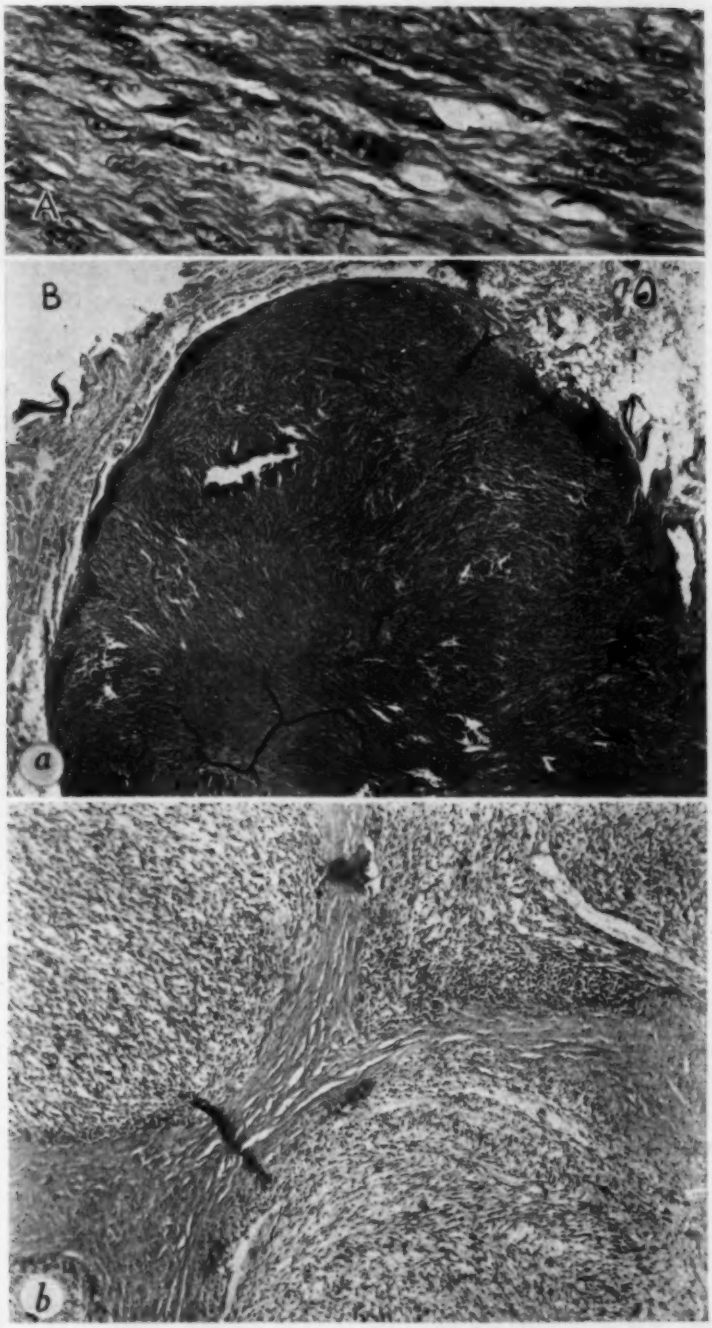

Fig. 4.—*A*, mitotic figure in a desmoid tumor. Notice the benign appearance of the surrounding fibroblasts (hematoxylin and eosin; × 415).

B, fibrosarcoma. (*a*) grade 4 encapsulation is shown (hematoxylin and eosin; × 18). (*b*) Fibrous septums have originated from the deep surface of the surrounding capsule (hematoxylin and eosin; × 40).

Cellularity.—When examining these connective tissue tumors under the low power, the microscopist will be immediately impressed by the degree of cellularity. When there is a paucity of cells, the eosinophilic collagen gives a distinct pinkish color to the section, whereas, when the lesion is cellular, the basophilic nuclei impart a bluish color. The cellularity of the desmoid tumors (table 1) was graded mainly 1 and 2, while that of the specimens of fibrosarcoma (table 2) was graded mainly 3 and 4. Figure 5*A* depicts grade 1 cellularity in a desmoid tumor, while figure 5*B* shows grade 3 cellularity in fibrosarcoma. The degree of cellularity, therefore, can be a distinct aid in determining the benignancy or malignancy of these tumors.

Mitosis.—At the outset of this microscopic study it was thought that the absence of mitotic figures would be the most important single factor in distinguishing a desmoid tumor from fibrosarcoma. Dockerty[32], in his study of ovarian fibroma, stated that "regardless of cellularity, the absence of mitotic figures in all but 2 per cent of ovarian fibromas substantiates a diagnosis of benign neoplasm, which is borne out by the subsequent clinical course of the patient." Bell[33] is quoted as follows: "There are gradual transitions between hard fibroma and grade 1 fibrosarcoma. When any mitoses are found and when the nuclei are surrounded by abundant cytoplasm the tumors should be considered fibrosarcoma." Table 1 shows the number of mitotic figures found on careful examination of sections of the desmoid tumors. Eleven of the 34 tumors, or approximately a third, showed mitotic figures. On the other hand, a glance at table 2 will show that mitotic figures were found in all the specimens of fibrosarcoma studied and that the number in each section was usually much higher than the number found in the desmoid tumors. Therefore, it can be deduced that the tumors not showing mitotic figures are "benign" and that those showing numerous mitotic figures are "malignant." However, in the present study, it is the transitional type of tumor, already alluded to, that is of most importance from the standpoint of an attempt at differentiation. The tumors showing infrequent mitotic figures cannot be placed in either the benign or the malignant class on the basis of this characteristic alone. Figure 4*A* shows a mitotic figure in a desmoid tumor, while figure 5*C* shows several mitotic figures in grade 2 fibrosarcoma.

Pathologic Mitosis.—Atypical or pathologic mitotic figures most commonly have the nuclear chromatin arranged in the form of a cross (quadripolar mitosis) or in T or Y formation (tripolar mitosis).

32. Dockerty, M. B.: Internat. Abstr. Surg. **81**:179, 1945.

33. Bell, E. T.: A Text-Book of Pathology, ed. 3, Philadelphia, Lea & Febiger, 1938, p. 253.

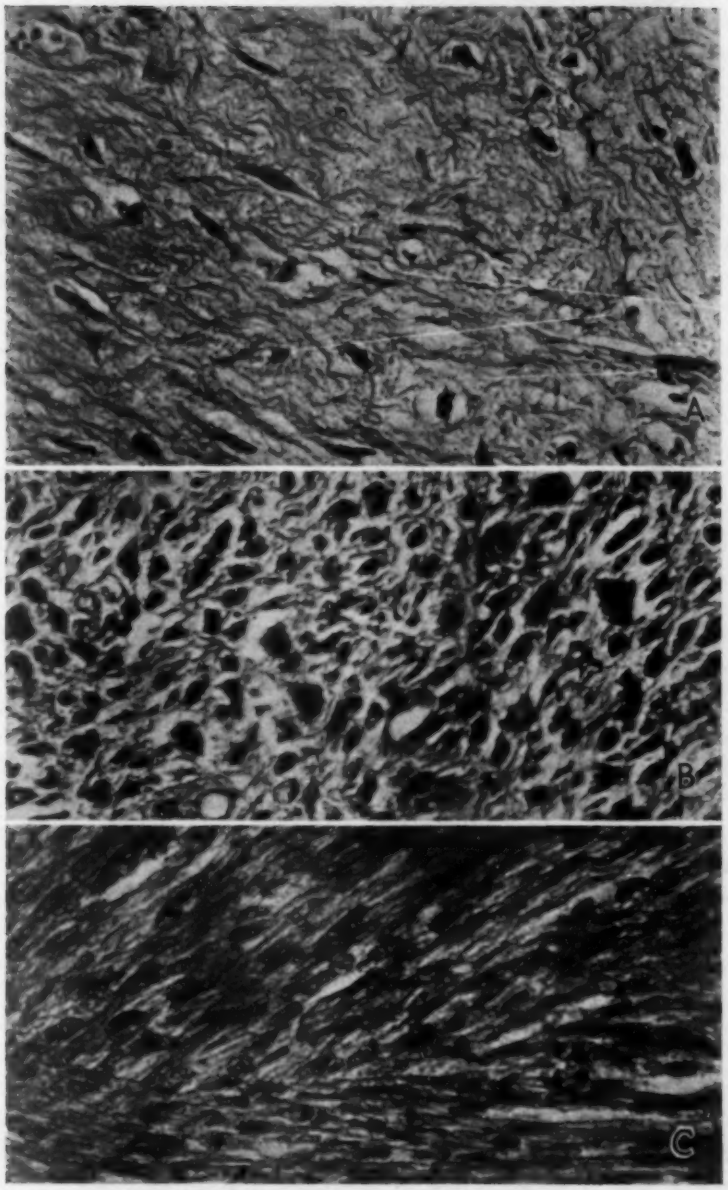

Fig. 5.—*A*, desmoid tumor. Note grade 1 cellularity and grade 1 variation in cellular size, shape, staining and nucleoli (hematoxylin and eosin; × 145).

B, fibrosarcoma. Note grade 3 cellularity and grade 3 variation in cellular size, shape, staining and nucleoli (hematoxylin and eosin; × 415).

C, mitotic figures in grade 2 fibrosarcoma (hematoxylin and eosin; × 415).

Occasionally the chromatin is scattered irregularly throughout the cell. The cytoplasm of the cell is usually more abundant than that of a cell which is undergoing normal mitosis. Hansemann[34], in his cytologic study of neoplasms in 1890, was the first to draw attention to the fact that "asymmetrical mitoses" occurred only in malignant tumors. Most investigators have agreed with regard to Hansemann's findings, Evans[35] stating that "atypical mitotic figures are unquestionably always a sign of a high grade malignancy." However, Bohrod,[36] in a recent paper, showed beautiful photomicrographs of "tripolar mitoses in non-neoplastic lesions" and stated that atypical mitotic figures are of no value in differentiating between the malignancy and benignancy. This article was shown to Broders,[37] who indicated that he held an opposite view. Broders preferred the term "pathologic mitosis" to the synonyms previously noted, for in his opinion a true pathologic mitotic figure is found only in a "malignant" lesion.

By reference to table 1 it will be noted that there were no pathologic mitotic figures found in any of the sections of desmoid tumor[5]. However, in 13 of the 34 specimens of fibrosarcoma (table 2) definite pathologic mitotic figures were found, varying in number from 1 to 15 per section (figs. 6*A* and *B*). Therefore, in a careful microscopic search, slightly more than a third of the malignant tumors diagnosed as low grade showed pathologic mitotic figures, while none were found in the benign tumors. In the microscopic differentiation between the borderline desmoid tumor and fibrosarcoma, the presence or the absence of pathologic mitotic figures would be of definite aid in about a third of the cases.

Tumor Giant Cells.—Boyd[38] gave an excellent description of the various types of giant cells. He divided them into three main classes: (1) tumor giant cells, (2) foreign body giant cells and (3) a miscellaneous group.

A tumor giant cell is a large cell containing one or several large, irregular hyperchromatic nuclei scattered throughout the cytoplasm of the cell (fig. 6 *C*). These asymmetric, dark nuclei give the cell a definite neoplastic appearance. The origin of these cells is not definitely known, but they are generally thought to be the result of direct (amitotic) division of the nucleus without division of the cytoplasm.

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34. Hansemann, D.: Virchows Arch. f. path. Anat. **119**:299, 1890.

35. Evans, N., in Collected Papers of the Mayo Clinic and Mayo Foundation, Philadelphia, W. B. Saunders Company, 1919, vol. 11, p. 349.

36. Bohrod, M. G.: Am. J. Clin. Path. **14**:563, 1944.

37. Broders, A. C.: Personal communication to the authors.

The characteristics of the foreign body giant cells, seen typically in tuberculosis, are well known and are of no concern in the present study.

In the miscellaneous group, Boyd[38] mentioned the Aschoff cell of rheumatism and the Sternberg-Reed cell of Hodgkin's disease. The multinucleated muscle giant cells (fig. 3 *B*), previously mentioned, may be placed in this category.

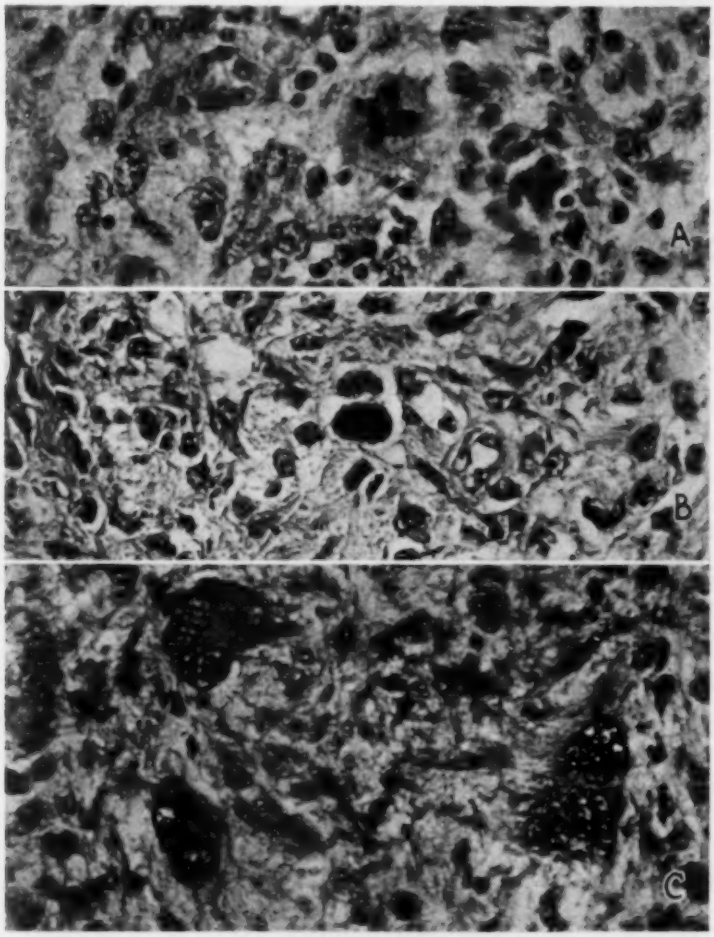

Fig. 6—*A*, cross-shaped quadripolar pathologic mitotic figure in grade 1 fibrosarcoma (hematoxylin and eosin; × 415).

B, Y-shaped tripolar pathologic mitotic figure in grade 1 fibrosarcoma (hematoxylin and eosin; × 415).

C, tumor giant cells in grade 2 fibrosarcoma. Note the large irregular, hyperchromatic nuclei scattered throughout the cells (hematoxylin and eosin; × 415.)

38. Boyd, W.: A Text-Book of Pathology: An Introduction to Medicine, ed. 4, Philadelphia, Lea & Febiger, 1943.

In this microscopic investigation no tumor giant cells were found in the sections of the desmoid tumors (table 1), but 22 of the 34 specimens of fibrosarcoma showed a varying number of tumor giant cells (table 2). From this finding one may conclude that a fibrogenic tumor containing true tumor giant cells is malignant.

Variation in Cellular Size, Shape, Staining and Nucleoli.—A comparison of tables 1 and 2 will show that the great majority of desmoid tumors presented little or no variation in these cellular characteristics (fig. 5 *A*), while 20 of the 34 specimens of fibrosarcoma showed a cellular variation graded 2 to 4 (fig. 5 *B*). Of the 14 remaining specimens of fibrosarcoma, 4 showed no variation and 10 showed grade 1 variation. These 14 tumors form an interesting group, for the uni-

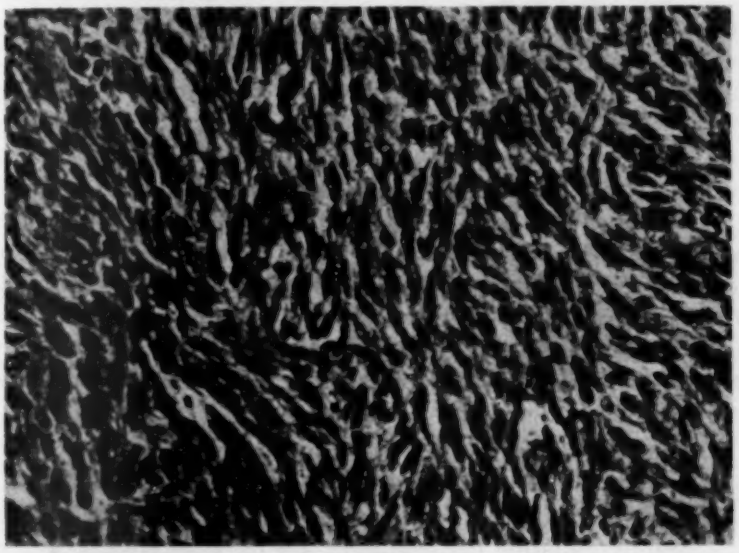

Fig. 7—Spindle cell fibrosarcoma showing grade 4 cellularity (hematoxylin and eosin; × 415).

formity of the cellular pattern is likely to suggest benignancy to the microscopist. However, they have other features which point toward malignancy, the main one being the degree of cellularity. Nine of the 14 neoplasms showed grade 3 or 4 cellularity (fig. 7). It is these highly cellular lesions with uniform cellular characteristics that Broders, Hargrave and Meyerding[31] classed as "cellular spindle cell fibrosarcomas." The cells increase by direct (amitotic) division; therefore, mitotic figures are scarce, and pathologic mitotic figures are rarely, if ever, seen. From these facts one can conclude that lack of variation in cellular size, shape and staining and in the nucleoli of the cells is, in

itself, of no benefit in determining whether a fibrogenic tumor is benign or malignant. On the other hand, if the variation is marked, one will favor the diagnosis of malignant tumor.

MICROSCOPIC DIFFERENTIAL DIAGNOSIS OF EXTRA-ABDOMINAL DESMOID TUMOR AND FIBROSARCOMA

As mentioned, the microscopic investigation in this study was carried out before reference to the clinical records was made. The critical examiner, in studying tables 1 and 2, will undoubtedly ask how the investigator knows for certain which neoplasm is a desmoid tumor and which is a fibrosarcoma. With use of the criteria presented in these tables there is no difficulty in diagnosing the definitely benign desmoid tumor and the definite fibrosarcoma. However, the borderline neoplasm falls into a different category. The differential diagnosis between a fairl ycellular benign fibrogenic lesion and low grade fibrosarcoma is one of the most difficult decisions a pathologist has to make. The main purpose of this investigation was to attempt to formulate the criteria which would be of help in this differentiation. The only way the pathologist can be sure, and not 100 per cent sure at that, of his diagnosis is to determine the final outcome in each individual case. The clinical records of the 34 cases of desmoid tumor and the 34 cases of fibrosarcoma were therefore examined, and follow-up letters were sent to all those patients for whom data were incomplete. Unfortunately, the results of the follow-up investigation were incomplete, owing to change of address, death in the interim and so forth (tables 3 and 4).

The microscopic observations shown in tables 1 and 2 correspond very well with the clinical results listed in tables 3 and 4. The microscopic features of the desmoid tumor and fibrosarcoma which are of value in differentiating between them are listed in table 5. The most important microscopic criteria of malignancy in a borderline fibrogenic tumor are pathologic mitotic figures, tumor giant cells, encapsulation and a high degree of cellularity, one or more of these features indicating malignancy. However, as in any microscopic diagnosis, it is the summation of all the microscopic detail that ultimately permits formulation of the diagnosis in the pathologist's mind.

DIFFERENTIAL DIAGNOSIS OF THE EXTRA-ABDOMINAL DESMOID TUMOR

Fibrosarcoma.—This is by far the most important tumor to differentiate from both a therapeutic and a prognostic standpoint. The microscopic differentiation has been thoroughly discussed in preceding pages. The gross pathologic appearance of the desmoid tumor has

TABLE 3.—*Therapy and Follow-up Report of Desmoid Tumors*

Case	Year of Onset	THERAPY			FOLLOW-UP	
		Year, or Years, of Operation	Irradiation			
			Year	Type	Year	Recurrence
1	1938	1939*, 1942*			1946	No
2	1915	1915*, 1918*			1946	No
3	1912	1915, 1917*			1925	No
4	1911	1912, 1914, 1916, 1917, 1919*	1917	Roentgen ray*	1946	No
5	1918	1918, 1920*, 1922			1922	Yes, tumor excised elsewhere
6	1945	1945*				No report
7	1922	1922*	1922	Radium	1930	No
8	1939	1939, 1941*	1939	Roentgen ray	1942	Yes
9	1924	1924, 1926*			1927	Yes?
10	1926	1927*			1945	No
11	1928	1929*			1931	No
12	1932	1932*			1936	No, patient died
13	1940	1940*				No report
14	1928	1932*, 1933*			1934	No
15	1934	1935, 1936, 1937*, 1940			1940	Yes, tumor excised elsewhere
16	1939	1940*, 1941* 1941*			1946	No
17	1937	1938, 1938, 1941, 1941*			1946	No
18	1940	1942*				No report
19	1928	1928, 1929, 1937, 1942*			1943	No
20	1942	1943*, 1944*	1943	Roentgen ray	1945	No
21	1942	1943*				No report
22	1943	1945*				No report
23	1934	1943*				No report
24	1918	1928, 1943*			1945	No, died
25	1941	1943, 1943*				No report
26	1944	1944, 1944*, 1946*	1944	Radium	1946	Ye s
27	1944	1944*			1945	No
28	1940	1944*			1945	No
29	1944	1944*				No report
30	1942	1943*				No report
31	1926	1926*, 1928*			1941	No
32	1922	1925, 1927, 1928				No report
33	1918	1921, 1922*	1922	Roentgen ray and radium*	1942	No, patient died
34	1926	1926, 1928*	1928	Roentgen ray	1946	No

*The therapy was given at the Mayo Clinic.

TABLE 4.—*Therapy and Follow-up Report of Tumors Diagnosed as Fibrosarcoma*

Case	Year of Onset	THERAPY			FOLLOW-UP			
		Year, or Years, of Operation	Irradiation		Year	Local Recurrence	Metastasis	Status of Patient
			Year	Type (Roentgen Ray, Radium)				
35	1910	1910, 1910*, 1911*			1912	+	+	Dead
36	1911	1912, 1914*, 1915*			1919	+	+	Dead
37	1910	1913*, 1915*			1915	0	+	Dead
38	1917	1919*, 1919*, 1920*, 1920*			1920	+	+	Dead
39	1903	1916, 1918, 1920*	1917 1919	Roentgen ray	1925	+	0	Dead
40	1916	1921*	1921*	Both	1923	+	+	Dead
41	1921	1921*, 1925*	1925*	Both	1930	+	+	Dead
42	1916	Biopsy 1924*			1926	+	+	Dead
43	1923	1925*			1946	0	0	Alive, well
44	1915	1928*, 1930*	1930*	Radium	1932	+	+	Dead
45	1915	1916, 1916*, 1916*			1917	+	+	Dead
46	1912	1914*, 1915*			1915	+	+	Dead
47	1914	1914, 1914*			1915	+	+	Dead
48	1918	1919, 1919*			1925	0	0	Alive, well
49	1918	1918, 1919, biopsy 1920*			1921	+	+	Dead
50	1906	1917, 1918, 1920*	1920*	Roentgen ray	1934	?	?	Dead
51	1917	Biopsy 1921	1921*	Roentgen ray	1921	+	+	Dead
52	1917	1917, 1918, 1920, 1922*	1922*	Both	1923	+	+	Dead
53	1913	Biopsy 1927*	1927*	Both	1927*	+	+	Alive, but died in 1928
54	1908	1926, 1928*	1926 1928*	Radium	1936	+	+	Dead
55	1925	1925, 1926, 1928, 1928*	1928*	Both	1939	+	+	Dead
56	1926	1926, 1927, 1928, 1929*			1935	+	+	Dead
57	1904	1926, 1928, 1929, 1929*			1931	0	+	Dead
58	1930	1930, 1930*			1931	+	?	Dead
59	1921	1921*	1921*	Both	1922	+	+	Dead
60	1918	1920, 1922*	1922*	Both	1924	+	+	Dead
61	1919	1920, 1921, 1922, 1923, 1924*, 1925*	1924* 1925*	Both	1926	+	+	Dead
62	1925	1925*	1925*	Roentgen ray	1934	0	0	Alive, well
63	1926	1926*	1926*	Radium	1934	0	0	Alive, well
64	1925	1926*, 1926*	1926*	Radium	1946	0	0	Alive, well
65	1925	Biopsy 1926*, 1927*	1926*	Both	1927	+	+	Dead
66	1929	1930, 1933*, 1933*	1930* 1932*	Roentgen ray	1946	0	0	Alive, well
67	1920	1922, 1930*	1930*	Roentgen ray	1935	?	?	Dead
68	1915	1925*	1925*	Roentgen ray	1946	0	0	Alive, well

*The therapy was given at the Mayo Clinic.

been discussed also. Fibrosarcoma differs as follows: (1) it tends to be larger, (2) it is usually round, (3) it is softer in consistency, (4) in more than 50 per cent of cases it is encapsulated, and in the remainder it is well circumscribed, (5) metastasis is common and (6) the surface usually has a salmon pink homogeneous appearance.

TABLE 5.—*Microscopic Characteristics of Thirty-four Extra-Abdominal Desmoid Tumors with Those of Thirty-four Tumors Diagnosed as Low Grade Fibrosarcoma*

Microscopic Characteristics	Extra-Abdominal Desmond Tumor		Fibrosarcoma	
Muscle inclusion	34		12	
Encapsulation	3		30	
Cellularity	Grade	Number of Cases	Grade	Number
	1	8	1	0
	2	22	2	7
	3	4	3	19
	4	0	4	8
Mitotic figures	11		34	
Pathologic mitotic figures	0		13	
Tumor giant cells	0		22	
Variation in cellular size, shape, staining and nucleoli	Grade	Number of Cases	Grade	Number
	1	7	1	9
	2	4	2	16
	3	0	3	4
	4	0	4	1
	No variation	23	No variation	4

Keloid.—Keloid is a fibrous tissue overgrowth in the skin caused by trauma, such as a blow, an infection or a laceration. The fibroblasts remain in the skin and subcutaneous tissues. They do not grow into striated muscle and incorporate the muscle fibers. However, if this fibrous overgrowth continues, the process may become neoplastic, with formation of a typical desmoid tumor.

Neurofibroma.—Neurofibroma is usually in the subcutaneous tissues and is softer in consistency than a desmoid tumor. Unlike the latter, it is usually freely movable. On microscopic examination the spindle-shaped cells are often found to be arranged in parallel groups, giving the palisade effect. The surrounding muscle is not engulfed.

Lipoma.—The lobulated, subcutaneous lipoma is readily differentiated clinically. However, the diagnosis is much more difficult when the tumor is situated deep to the fascia. During surgical removal of such a tumor, its nature is readily recognized by the gross characteristics.

Dermatofibroma.—This benign neoplasm, like the keloid, remains in the deeper layers of the skin and subcutaneous tissues and is usually not more than a centimeter in diameter. The fibroblasts, of which it is composed, are arranged like spilt matches. An occasional normal mitotic figure may be seen. The erector pili muscle cells may be seen at the periphery of the tumor.

Diffuse Subacute Myositis.—Inflammation in muscle may cause considerable fibrosis, giving the appearance of a fibrogenic neoplasm with engulfed striated muscle. In this instance, however, the inflammatory cells are diffusely scattered throughout the lesion, while in a desmoid tumor the round cell infiltration is seen only around the periphery of the growth, as previously described (fig. 3*B*).

Benign Rhabdomyoma.—This is a tumor of striated muscle cells. The differentiation from a desmoid tumor is mainly on a microscopic basis, the constituent cells being larger than fibroblasts; by carefully scrutinizing it with subdued light, one may see cross striations. These may be much more readily visualized if the section is stained with phosphotungstic acid.

Hemangioma and Hemangioendothelioma.—These two neoplasms of endothelial origin may grow into striated muscle and engulf fibers in much the same manner as does a desmoid tumor. Microscopically, muscle inclusion is the only feature these tumors have in common with the desmoid tumor. The clear, polyhedral cells are arranged in groups or sheets around central vessels, the groups being separated by varying amounts of muscle and fibrous tissue. The benign hemangioma differs from the malignant hemangioendothelioma in that it has larger blood spaces and is less cellular, the cells do not vary appreciably in size, shape and staining characteristics and mitotic figures are rarely seen.

TREATMENT OF THE EXTRA-ABDOMINAL DESMOID TUMOR

At the present time there are three forms of therapy to be considered in treating a desmoid tumor: radical local excision, radium and roentgen therapy and endocrine therapy.

Radical Local Excision.—A desmoid tumor is a benign tumor having the malignant property of local invasion. Therefore, for complete eradication of this neoplasm a wide, or more appropriately, a radical excision has to be carried out. However, if the lesion occurs adjacent to, or surrounds important structures, such as major blood vessels or nerves, a more conservative type of surgical procedure will be indicated. The penalty for conservatism is a very high rate of recurrence. The surgeon will have to weigh carefully the value of radical versus conservative operation in such a case.

Radium and Roentgen Therapy.—This type of treatment should never be used for a lesion that can be radically excised. If the tumor can be excised as a whole and the microscopic observations corroborate the diagnosis of desmoid tumor, there is no indication for radium or roentgen therapy.

In the present study, radium or roentgen therapy or both were used in those cases in which either only incomplete removal or no removal at all was possible on account of the location of the tumor. There is no direct evidence that this type of therapy appreciably affected the growth of the neoplasm. However, intensive roentgen ray or radium therapy will probably be continued in such cases in the hope that the rate of growth of the tumor will be checked to some extent.

Endocrine Therapy.—From the standpoint of treatment, this is the most recent addition to the list of available procedures. The rationale of this type of therapy was thoroughly discussed in connection with the etiologic consideration of the desmoid tumor. Radiation castration and the so-called antifibromatogenic sterols, testosterone, progesterone and desoxycorticosterone, have not, to date, been given a sufficient clinical trial to indicate whether they will prevent further growth or cause regression of desmoid tumors. This form of therapy should be reserved for those desmoid tumors which are inoperable or can be only partially removed. Endocrine therapy may be of definite benefit in treating these unfortunate persons.

PROGNOSIS

In the early medical literature, desmoid tumors were called "recurring fibroid tumors,"[4] for they almost invariably recurred. This was due to the fact that the surgeons in the midnineteenth century did not practice wide excision of the lesion. The rate of recurrence for cases of desmoid tumor of the abdominal wall has ranged from 10 to 40 per cent[39]. This high rate of recurrence has been due to (1) the inability of the surgeon to remove the growth completely, owing to its nature and location, and (2) the fact that many surgeons have not realized that only a radical local excision will ablate this lesion.

The results of therapy administered elsewhere and at the Mayo Clinic for the 34 extra-abdominal desmoids investigated in this study are given in table 3. It will be noted that 20 of the 34 patients underwent two or more operations and that 7 of the 20 were operated on two or more times at the clinic. As to the remaining 14 patients, who were operated on only once, there were no follow-up reports on 8, no

39. Pfeiffer,[6] Pearman and Mayo.[18]

recurrence in 4 during two, four, eight and eighteen years, respectively, and the remaining 2 were operated on in 1944, with no recurrence known one year later.

After a careful perusal of the clinical records and follow-up data in these cases of extra-abdominal desmoid tumor, it was noted that if the tumor recurred after therapy, it did so usually in the first six months and in every case within the first year. Of the 26 desmoid tumors followed one year or longer in the present series, 20, or 77 per cent, recurred one or more times.

REPORT OF CASES

Three typical cases of desmoid tumor are reported herein. The case numbers which follow correspond to those in tables 1 and 3.

Case 1.—A woman 50 years of age reported to the clinic in January 1939, complaining of a lump on the upper lateral aspect of her left arm. She had first noticed the lump six months prior to admission. On examination the lump was found to measure about 5 by 5 cm. and to be attached to the deep structures. The examining doctor thought it was probably a malignant tumor of the left humerus. The roentgenograms of the left humerus did not reveal any abnormality. After roentgenologic examination the patient was seen by an orthopedic consultant, who thought the tumor was probably a benign fibroma. The tumor was excised widely; it was diagnosed as grade 1 fibrosarcoma by the pathologist. Ten months after the operation the patient again noticed some discomfort in her left shoulder and thought she felt some thickening in the upper end of the scar. When she returned to the clinic in July 1942, there was a mass the size of an orange in the lower deltoid region. The mass was widely excised along with three fourths of the deltoid muscle; the pathologic diagnosis was desmoid tumor.

In August 1946 this woman was alive and well, with no evidence of recurrence of the tumor.

Sections of the neoplasm removed in both 1939 and 1942 were examined and found to have the microscopic characteristics noted in table 1. There was an average of 7 normal mitotic figures per section but none of the other criteria of malignancy was present. If this tumor was malignant, it probably would have occurred in the succeeding four years.

Case 12.—A 37 year old woman came to the clinic in April 1931, at which time she underwent radical mastectomy for grade 3 adenocarcinoma of the breast.

In July 1932 she returned for removal of an acorn size lump that had gradually formed in the scar of the previous operation. Clinically the lump was thought to be a recurrence of the carcinoma. At the time of operation it was noted that the growth involved the intercostal muscles. The pathologist made a diagnosis of desmoid tumor.

The patient died of carcinomatosis in 1936, there being no evidence of recurrence of the desmoid tumor.

This case illustrates the formation of an extra-abdominal desmoid tumor at the site of a previous operation.

Case 17.—In 1937, at the age of 21 years, this patient was having her hair cut, when the barber noticed a lump on the back of her neck just below the occiput. This lump was removed and was diagnosed as neurofibroma by the pathologist. The growth recurred in a few months and was again excised in 1938; this time a diagnosis of fibroma was made. The patient noticed a recurrence in about three months, and the growth became progressively larger. In March 1941, tissue for biopsy was taken; a diagnosis of neurogenic sarcoma was reported. These sections were sent to one of the pathologists in the clinic, who reported that they showed low grade fibrosarcoma. However, he wanted another opinion and, consequently, another clinic pathologist examined the slides and called the lesion a desmoid tumor.

On being examined at the clinic the patient was found to have a firm, fixed tumor the size of a large orange immediately below the occipital region. A wide excision of the tumor (fig. 2) was carried out in June 1941, the dissection being so radical that the patient left the hospital wearing a neck brace. A third clinic pathologist examined this specimen and again a diagnosis of desmoid tumor was made.

As of August 1946, this young woman was known to have little or no malfunction of movement of the cervical part of the spinal column and no evidence of tumor formation.

This case amply shows how difficult the microscopic diagnosis of a desmoid tumor can be and also clearly demonstrates that radical operation is the cure for this type of neoplasm.

SUMMARY AND CONCLUSIONS

A detailed clinicopathologic study of 34 cases of extra-abdominal desmoid tumor has been presented. A similar number of cases of low grade fibrosarcoma have been studied in an attempt to formulate criteria which will aid in the microscopic differentiation of the two tumors.

The extra-abdominal desmoid tumor is the same pathologic entity as the desmoid tumor occurring in the musculoaponeurotic structures of the abdominal wall.

The desmoid tumor is a benign fibrous neoplasm, which has the peculiar characteristic of locally invading and destroying the adjacent striated muscle. It does not metastasize, and there is no evidence in the present study that it undergoes sarcomatous change.

Two theories of origin have been presented, namely, that of a relationship to trauma and that of an endocrinologic basis. Forty-one per cent of the 34 patients definitely linked the onset of the tumor with some previous trauma. However, only 3 of the 34 tumors showed a trace of hemosiderin on microscopic examination. Thus, the histories tend to corroborate the traumatic etiologic basis, while the microscopic observations tend to disprove it.

There is strong evidence that the endocrine glands may play a part in the origin and "physiology" of these tumors.

The desmoid tumor is rare, but if the surgeon and the pathologist will keep it in mind, it will no doubt be found more frequently in the future than it has been in the past.

The extra-abdominal desmoid, like its counterpart in the abdominal wall, is found more often in the female, the ratio in the present series being 2.4:1.

In an attempt to differentiate between the desmoid tumor and low grade fibrosarcoma, the following microscopic criteria are important: (1) Encapsulation: A well circumscribed, encapsulated fibrogenic tumor should be considered malignant until proved otherwise. (2) Cellularity: An acellular fibrous tumor will usually be benign, while a high degree of cellularity definitely points toward malignancy. (3) Mitotic figures: Fibrogenic tumors not showing mitotic figures are benign, while those with numerous mitotic figures are malignant. However, tumors showing infrequent mitotic figures cannot be placed in either the benign or the malignant class on the basis of this characteristic alone. (4) Pathologic mitotic figures: One or more pathologic mitotic figures indicate malignancy. (5) Tumor giant cells: These cells are found only in the malignant fibrogenic lesions. (6) Variation in cellular size, shape, staining and nucleoli: As the variation in these cellular features increases so does the tendency toward malignancy.

When at all possible, desmoid tumors should be treated by radical excision. It is doubtful whether roentgen rays and radium are of value in treating them, but when this form of therapy is used, it should be given in intensive doses.

Endocrine therapy holds out a ray of hope in the inoperable cases, but there is much to be learned about this form of therapy.

The rate of recurrence in the 34 cases of extra-abdominal desmoid tumor under review was high, calling for a reassessment of the surgical and radiation forms of therapy and for more research in, and clinical trial of, endocrine therapy.

CHONDROMYXOID FIBROMA OF BONE

A Distinctive Benign Tumor Likely To Be Mistaken Especially for Chondrosarcoma

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WE HAVE occasionally encountered a rather distinctive benign tumor of bone whose anatomic peculiarities seem best expressed in the name "chondromyxoid fibroma." The lesion first impressed itself on us when we were reviewing for publication our material on chondrosarcoma. Among the tumors classified in our files under the latter heading we found 3 of the type under discussion here, and we made brief mention of these in our paper on chondrosarcoma[1]. Since then we have encountered 5 additional cases of the lesion, our total experience with it to date thus comprising 8 cases.

In its gross appearance, the tissue of the tumor bears a certain resemblance to cartilage, though it is somewhat more firm than, and not as glistening as, hyaline tumor cartilage. Microscopically, too, the tissue of a particular specimen may simulate cartilage tissue in one area or another, thus justifying the qualification "chondroid." However, in some specimens one may fail to find such areas at all, and in any case the tumor tissue fundamental to the lesion is clearcut in its deviation from hyaline tumor cartilage tissue. Indeed, the basic cytologic picture of the lesion is that of a peculiarly differentiated connective tissue tumor whose cells lie loosely in a myxoid intercellular matrix which, in one or another tumor or tumor area, may undergo substantial collagenization. The presence, in the tumor tissue, of smaller or larger numbers of cells exhibiting nuclear atypism may give the cytologic picture of the lesion a false cast of ominousness, explaining why the lesion may come to be misinterpreted as a cancer.

Search of the medical literature fails to reveal, under whatever title, a clearcut clinicoanatomic description of the lesion under discussion, based on adequate experience with it. In the past, some sporadic cases of this kind have probably been reported as instances of chon-

From the Laboratory Division, Hospital for Joint Diseases.

1. Lichtenstein, L., and Jaffe, H. L.: Am. J. Path. **19**:553, 1943.

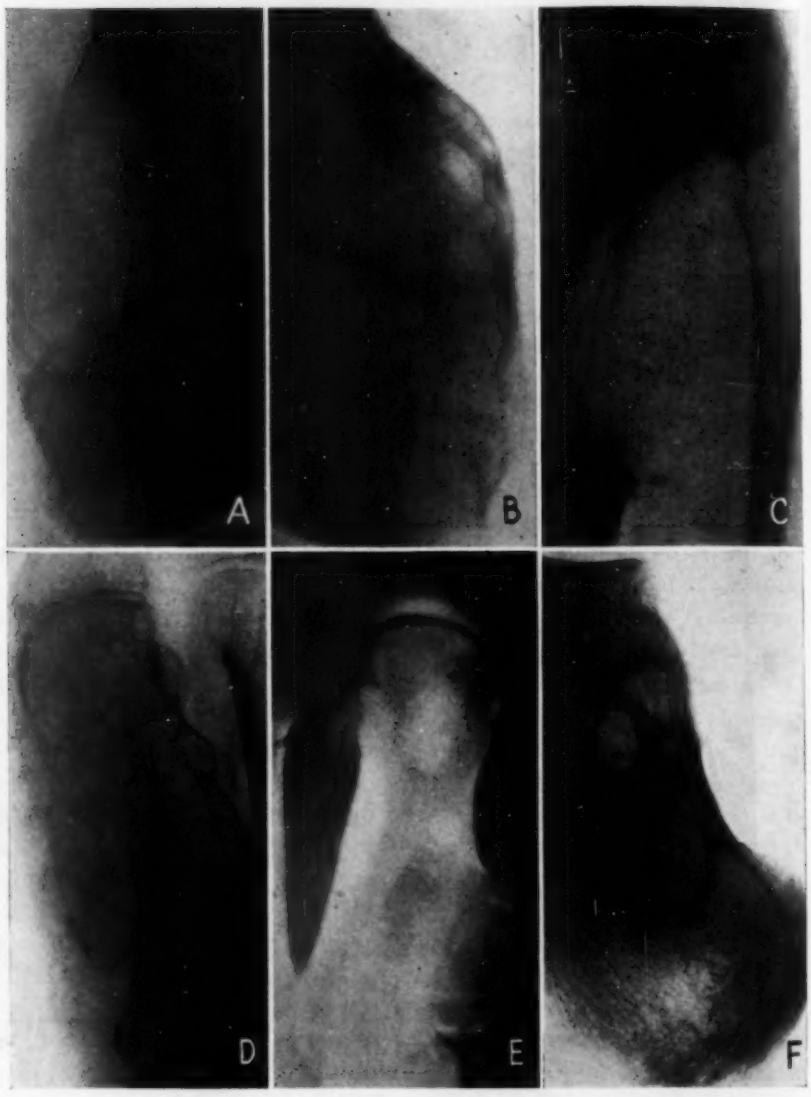

FIG. 1.—*A*, roentgenogram showing a relatively large ovoid lesion in the lower mataphysis of a femur of a girl of 17. The lesion has a characteristic eccentric location. The contour of the affected area of the bone is somewhat bulged, and the expanded contour is outlined in part by a thin shell of bone. Internally the lesion is demarcated by a border of sclerosed bone. The area of the lesion appears radiolucent. The patient had complained of pain in the outer aspect of the knee for three years prior to surgical intervention. From the surgical specimen it was thought at first that we were dealing with enchondroma of an aggressive type, an impression subsequently rectified in the light of further experience with the lesion. The patient has been followed for six years, during which time there has been no indication of recurrence.

B, roentgenogram showing a somewhat smaller and more roundish but comparable lesion in the lower metaphysis of a femur of a boy of 18. This patient has shown no sign of recurrence after curettage for over seven years.

(Legend continued on next page)

drosarcoma. Very likely some lesions reported as "myxoma of bone" also represent cases in point. However, study of the papers dealing with myxoma of bone is, on the whole, unrewarding. Certainly, the older papers,[2] lacking roentgenographic and even adequate cytologic illustrations, do not permit one to maintain with any certainty that a given lesion described as myxoma is actually chondromyxoid fibroma. This relevance is difficult to establish even in regard to individual cases reported more recently, under such names as "myxoma,"[3] "central myxoma,"[4] and "embryonal enchondroma"[5] of bone.

CLINICAL CONSIDERATIONS

Age and Sex Incidence.—As to the ages of our 8 patients on admission to the hospital, 4 were in their 'teens (ages 13, 17, 18 and 18), 2 were in the twenties (ages, 22 and 28) and 2 were older adults (ages, 42 and 56). As to sex, 4 were males and 4 females.

Localization.—So far we have encountered the lesion only in one or another bone of a lower limb, but it is to be doubted that this is its exclusive location. As to our 8 cases in particular, in 3 it was in a tibia, in 2 in a femur, in 2 in a metatarsal bone, and in 1 in a calcaneus. Within the femur or the tibia, the lesion was found consistently in the metaphysial area adjacent to the knee joint. Also, in

2. Soubeyran, P.: Rev. de chir. **29**:239 and 588, 1904.
3. Copello, O.; Bol. y trab. de la Soc. de cir. de Buenos Aires **9**:1151, 1935.
4. Herfarth, H.: Arch. f. klin. Chir. **170**:283, 1932.
5. Freund, E.: Arch. Surg. **33**:1054, 1936 (case 1).

(EXPLANATION OF FIGURES CONTINUED FROM PAGE 542)

C, roentgenogram showing a relatively large radiolucent lesion in the upper metaphysis of a tibia. The lesion is sharply demarcated internally by a sclerosed border. Externally the contour of the affected bone area is bulged, and a delimiting shell of bone is visible only at the lower end of the lesion. The patient was a girl of 18, who gave a history of having been kicked on the leg six months previously.

D, roentgenogram showing a lesion in a first metatarsal bone, which has expanded and replaced the entire bone except for a small portion of the head. The patient was a man, 56 years of age, who had complained of intermittent pain for about three years. Treatment consisted of curettage and insertion of an inlay bone graft. The result was entirely satisfactory, and there has been no indication of recurrence for the past three and one half years.

E, roentgenogram showing a relatively small and still eccentric lesion in the proximal or juxtaepiphysial area of the shaft of a first metatarsal bone. It does not extend across the full width of the shaft and otherwise resembles the localization in the long bones. The tissue curetted from the lesion was originally interpreted as showing chondrosarcoma (this was the first instance of the lesion that we encountered), but the patient, a young man of 28, showed no evidence of recurrence during the ensuing three years.

F, roentgenogram showing a well demarcated lesion in a calcaneus, which was eccentrically located in the volar part of the body, at some distance from the calcaneal apophysis. The patient was a boy of 18, whose complaints were of relatively short duration. Only a few months have elapsed since the time of operation, but the clinical result is thus far entirely satisfactory.

these bones, the lesion did not extend across the width of the metaphysis, but did erode and even completely destroy the local cortex (fig 1 *A*, *B* and *C*). In regard to involvement of a metatarsal bone, the location in the 2 pertinent cases was in a first metatarsal. In one of these the lesion was in the proximal or juxtaepiphysial area of the shaft, did not extend across the full width of the shaft, and otherwise resembled the localization in the long bones (fig. 1 *E*). In the other case the lesion occupied and modified the entire metatarsal bone except for a small portion of the head (fig. 1 *D*). In the calcaneus the lesion was also eccentrically located in the volar part of the body, definitely at some distance from the calcaneal apophysis (fig. 1 *F*).

Clinical Complaints.—The complaints were usually mild, and were ordinarily of at least some months' standing before the patient sought admission to the hospital for treatment. Consistently, there was some pain, which was intermittent and not very distressing. Most of the patients had already been aware, for some time, of the presence of a palpable mass at the affected bone site. As with bone tumors in general, there was in several cases a history of a previous injury, but in no case could any causal connection between this injury and the onset of the lesion be proved.

GROSS PATHOLOGIC ASPECTS AND ROENTGENOGRAPHIC APPEARANCE

Though simulating cartilage tumor tissue grossly, the tissue of chondromyxoid fibroma lacks the blue-white luster and the faceted pattern of the former. In the main it is whitish, rather glistening and firm but compressible. Also, despite the myxoid character of the intercellular matrix, the tissue does not appear slimy in the gross.

The tumor destroys the bone at the site of its growth, and residua of the original spongy trabeculae are not usually found within it. Where it abuts on the cortex, it tends to erode and destroy this. As the original cortex gives way, the contour of the affected area of the bone becomes bulged. The expanded contour may be outlined, in part or throughout, by a thin shell of bone newly deposited by the periosteum. However, where a demarcating cortical shell is absent, gross examination will reveal that the tumor is still contained by the periosteum and the overlying parosteal connective tissue. That is, the tumor tissue does not violate the bounds of the periosteum. Furthermore, as noted, the tumor usually does not extend across the entire width of the bone at the site of its growth. Along its inner surface, one usually finds it bordered by a smaller or larger zone of sclerosed and often distinctly grooved osseous tissue.

These various gross features of the lesion find their reflection in its roentgenographic picture (fig. 1). First, it is to be noted that the

tumor is usually of appreciable size. Also, though located by predilection in a metaphysial area of a tubular bone, it ordinarily does not encroach on the epiphysial end proper. While the disease focus may appear roundish, it is usually ovoid in shape. Its long axis is usually in the long axis of the affected bone. In one or another instance it has measured as much as 7 or 8 cm. in its longest dimension and 4 or 5 cm. across.

In conformity with the absence of osseous trabeculae in the tumor tissue, the area of the lesion appears, on the whole, radiolucent. Since its advance in the interior of the bone is associated with the development of a sclerosed perifocal osseous margin, which may be distinctly grooved, the area of the lesion may also present coarse trabeculation roentgenographically. The external border may be demarcated roentgenographically throughout by a more or less expanded thin shell of bone, or it may be difficult to visualize in places because no periosteal cortical shell has been laid down there.

On the whole, the roentgenographic picture has a certain distinctiveness, at least when the lesion is in a long bone and has attained appreciable size. However, even when it is in a long bone and certainly when in other sites, the clinical recognition of the lesion on the basis of its roentgenographic appearance may be difficult. In particular, one may have to consider, in relation to differential diagnosis, the possibility of a focus of fibrous dysplasia, a rather atypical unicameral bone cyst or even enchondroma. The likelihood of confusing chondromyxoid fibroma with giant cell tumor roentgenographically is remote.

MICROSCOPIC STRUCTURE

The cytologic picture presented by chondromyxoid fibroma in an individual case is accented by what one may interpret as the degree of maturation. In the least mature tumor one notes, characteristically, fields of tumor cells lying loosely within a myxoid intercellular matrix and demarcated peripherally into pseudolobules by narrow tracts of more closely compacted tumor cells. Even in the more mature tumor a tendency toward lobular arrangement is maintained. Increasing maturity of the tumor is associated with progressive collagenization of the intercellular matrix. Specifically, one observes, in some parts of an individual lesion or throughout it, a loose network of criss-crossing collagen fibers. In other lesions the collagen fibers, more numerous and compact, are found interwoven into a dense mat, though smaller or larger hyaline patches may even appear (fig. 4 *A*). At the same time, in parts of an individual lesion, or almost throughout it, the matrix may come to take on a chondroid appearance, and since, fur-

thermore, many of the tumor cells come to lie in lacunas, such tissue as a whole acquires an aura of cartilage (fig 4 *B*).

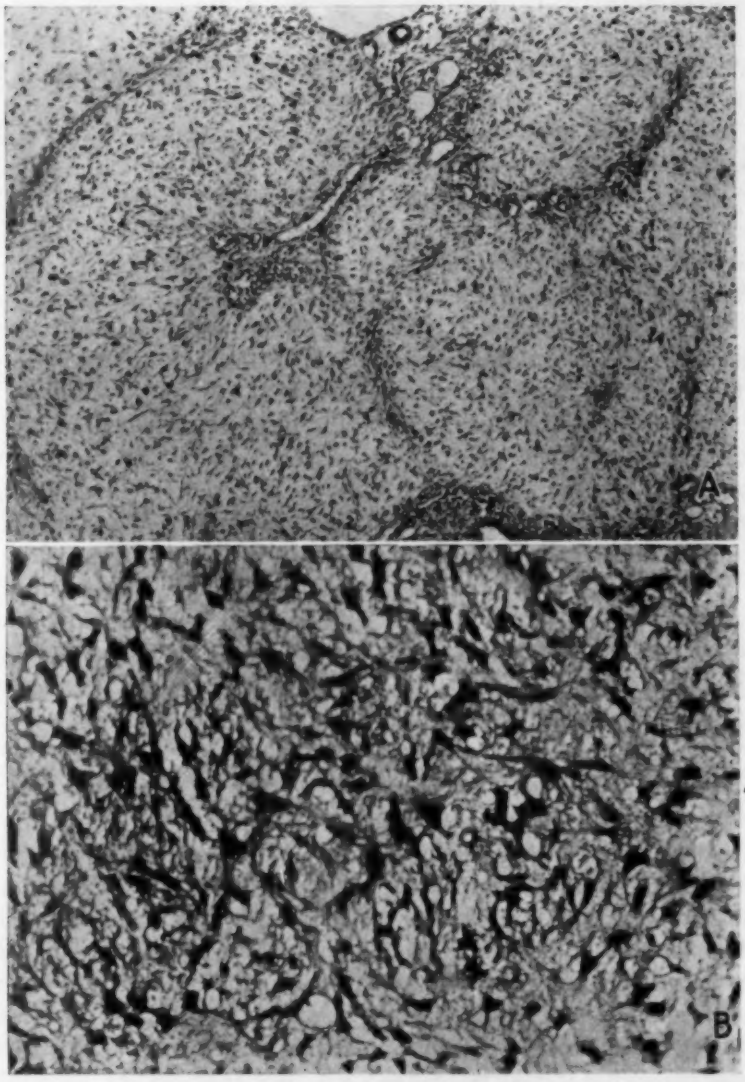

FIG. 2.—*A*, photomicrograph showing the general cytologic pattern of a lesion which has undergone relatively little collagenization. Fields of spindle-shaped tumor cells lying loosely within a myxoid intercellular matrix are demarcated into pseudolobules by narrow vascularized tracts of more compact tumor cells. The tissue is from the lesion illustrated in figure 1 *F*. Mallory's stain; × 50.

B, photomicrograph showing a higher magnification of tissue from the lesion illustrated in *A*. The tumor cells have indistinct cytoplasmic borders, though many of the cells show fibrillar cytoplasmic processes. The intercellular matrix is vacuolated and has a basophilic hue when stained with hematoxylin and eosin. × 225.

Within fields in which the matrix is myxoid, the tumor cells, on the whole, have only indistinct cytoplasmic borders, though many of the cells show branching fibrillar cytoplasmic processes. The cell

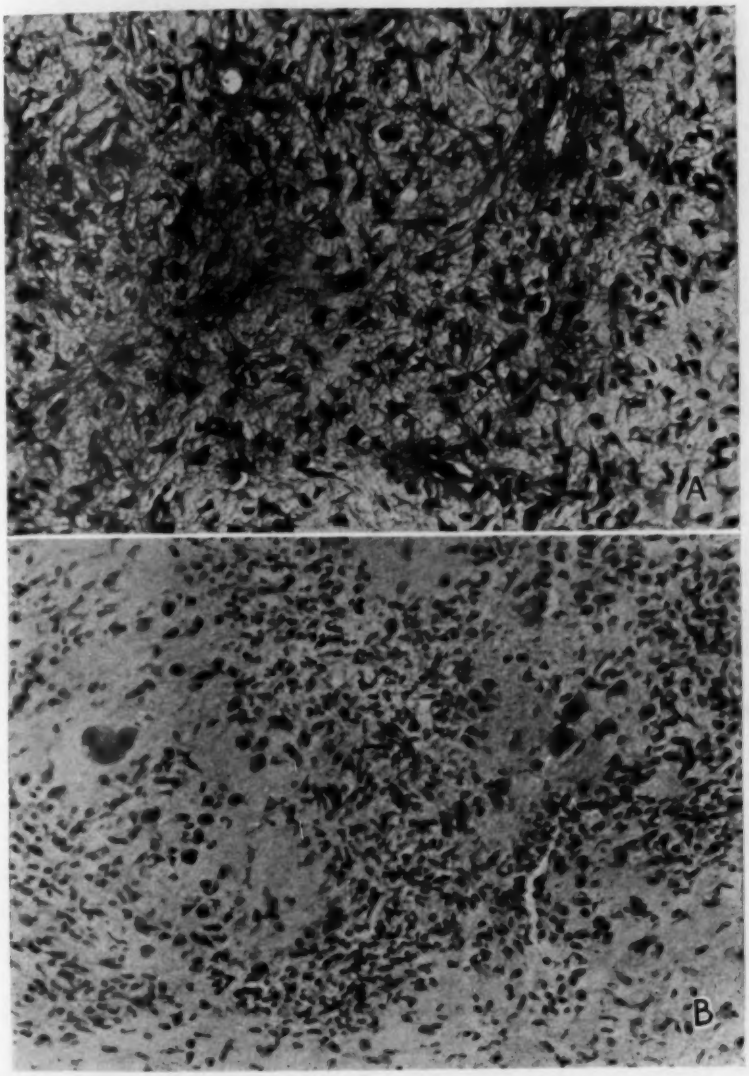

FIG. 3.—*A*, photomicrograph of a field of the lesion illustrated in figure 1 *D*. As compared with the tissue of the lesion illustrated in figure 2 *B*, this shows the tumor cells somewhat more compacted and the intercellular matrix substantially richer in collagen fibers. × 225.

B, photomicrograph of tumor fields from the lesion illustrated in figure 1 *E*, showing a nest of cells with prominent hyperchromatic nuclei and a cell with three such nuclei. It is the appearance of such fields that may cause the lesion to be overdiagnosed as a cancer. × 150.

nuclei are mainly ovoid or multipolar and are rather prominent. The supporting intercellular material is vacuolated and has a bluish hue when stained with hematoxylin. However, when sections are stained

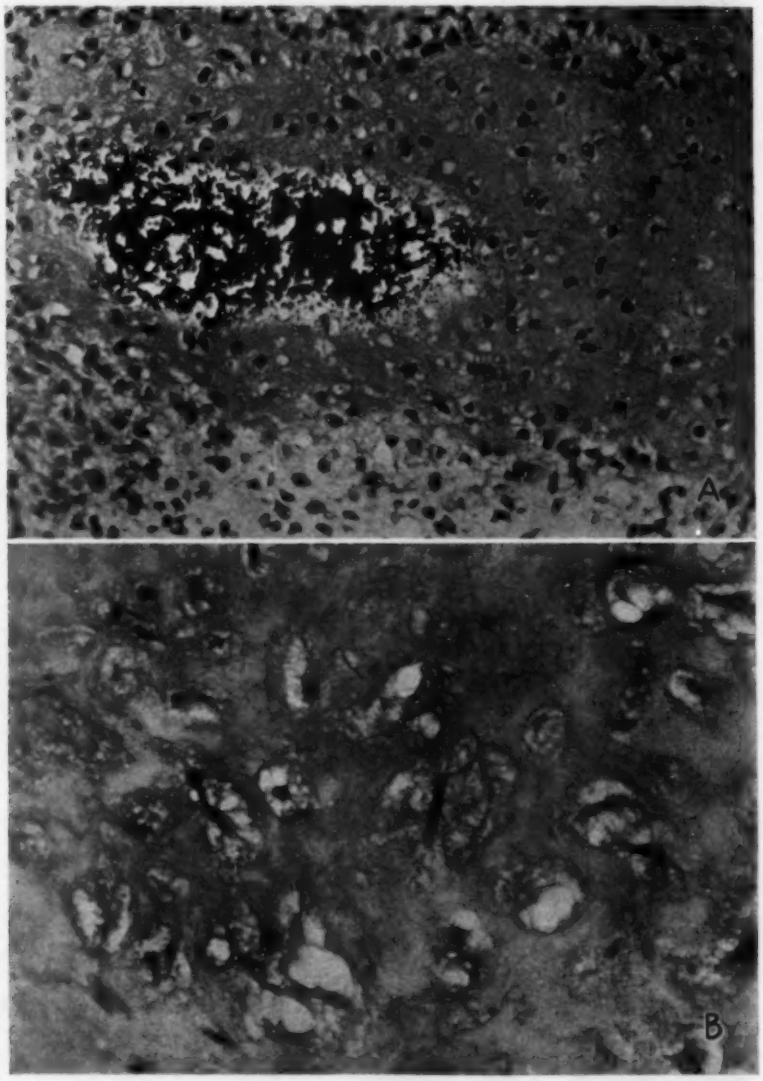

FIG. 4—*A*, photomicrograph of a field from the lesion illustrated in figures 1 *D* and 3 *A*, showing an area in which the intercellular matrix has undergone hyaline transformation and calcification. Many of the tumor cells within this focus are roundish and come to lie within lacunar spaces. Fields such as this are not a conspicuous feature of the lesion, and often one has to search for them. × 225.

B, photomicrograph of a more mature, collagenized lesion showing a tumor field in which the matrix and also the tumor cells come to take on a chondroid appearance. A substantial part of the lesion was of this character. × 400.

for the demonstration of mucin, the intercelullar matrix does not give the mucin response. Indeed, it seems that the myxoid character of the matrix is attributable rather to its aqueous content than to mucin. Furthermore, under appropriate staining, such fields of tumor tissue fail to present evidence of fat in the matrix (fig. 2 *A* and *B* and fig. 3 *A*).

At the periphery of the lobules, where the tumor cells are more closely compacted, one is likely to find tumor cells with particularly prominent nuclei. Such cells may present large plump nuclei, strikingly hyperchromatic nuclei, and atypical large double or multiple nuclei fig. 3 *B*). At the periphery of the lobules one usually also notes vascular channels. About these vessels, as also around the vessels carried in by tracts of supporting connective tissue, one may observe evidence of blood extravasation. Also, one may see some multinuclear giant cells and hemosiderin-laden macrophages, a sprinkling of small mononuclear cells, and occasional polymorphonuclear leukocytes. There may also be some macrophages containing sudanophilic droplets, and, occasionally, small nests of foam cells. Among the other secondary changes, focal calcification and ossification may be noted. But few lesions show any evidence of them, and it is only exceptionally that they are found in more than an occasional small focus.

That chondromyxoid fibroma is likely to be misdiagnosed as chondrosarcoma or at least enchondroma of an aggressive type has already been intimated. This tendency stems in part from the suggestive cartilaginous character of the tumor tissue in the gross. It is furthered by the cytologic appearance of the intercellular matrix, but more specifically by the presence in the tumor of smaller or larger numbers of cells with large nuclei, double nuclei or even bizarre nuclei. By the same token, when the intercellular matrix is less obviously chondroid and is predominantly myxoid, one can readily understand also that a diagnosis of myxosarcoma may sometimes be entertained. However, despite cytologic features which seem to be ominous, we know that the lesion is entirely benign, a curettage which does not even completely remove it not being followed by recurrence.

One may logically raise the question whether the lesion under discussion is possibly related to so-called myxoma of bone. Certainly, as noted, the tissue of chondromyxoid fibroma is not at all mucoid or gelatinous in its gross appearance and does not give, under appropriate staining of tissue sections, the mucin reaction which one expects of myxomatous tissue. If one compares the tissue of the tumor with the tissue of an umbilical cord, one finds, of course, but little resemblance between them. However, that the tumor is sometimes misconstrued as pure myxoma or some sort of myxoma of bone seems

highly probable. On the other hand, the meager experience we have had with the lesion called myxoma of the jaw bone shows that, whatever the latter lesion may actually represent, its cytologic picture does not correspond to that of the lesion we call chondromyxoid fibroma of bone.

PROGNOSIS AND TREATMENT

As already noted, the lesion is benign and does not tend to recur even after mere curettage. Thorough curettage of a large lesion may make filling of the bone defect with bone chips or a bone graft desirable. We have corroborative follow-up records in 6 of our 8 cases. In these, the follow-up has extended respectively over seven, six, three and one-half, three, two and one and one-half years, and in none has there been a local recurrence. In one of the remaining cases, the curettage was done only several months ago, and the follow-up is thus too short for evaluation, but clinically the result is entirely favorable so far. The other remaining case has been lost from sight.

SUMMARY

The tumor described and called chondromyxoid fibroma of bone seems not to have been generally recognized in the past as a distinctive neoplasm, although it appears likely that in single instances it has been reported as enchondroma and myxoma, or their cancerous counterparts. Our interpretation of the lesion is that of a peculiarly differentiated connective tissue tumor exhibiting in the course of its evolution certain chondroid and also myxoid traits which hallmark the lesion cytologically. It is composed basically of cells lying loosely in a myxoid intercellular matrix which, as the tumor matures, may undergo substantial collagenization. The tissue of any particular specimen may also come to simulate cartilage tumor tissue in some or many fields, and in its gross appearance it likewise bears a certain resemblance to cartilage. The presence of smaller or larger numbers of tumor cells exhibiting nuclear atypism may cause the lesion to appear more ominous than we know it to be, explaining why it may come to be overdiagnosed as a malignant tumor, particularly as chondrosarcoma.

We have encountered the lesion thus far only in one or another bone of a lower limb—specifically, in the femur, the tibia and some of the bones of the foot. Within the femur or the tibia, the lesion was found consistently in the metaphysial area adjacent to the knee joint. Also, in these bones, the lesion did not extend across the entire width of the metaphysis, but did erode and even substantially destroy the local cortex, causing the contour of the affected area of the bone to

become bulged. In most instances the expanded contour was outlined in part or throughout by a thin shell of bone newly deposited by the periosteum. Where a demarcating cortical shell was absent, it was evident that the tumor was still contained by the periosteum and the overlying parosteal connective tissue. Along its inner surface the tumor is usually bordered by a zone of sclerosed bone. Within the bones of the foot the lesion likewise displayed a tendency to be well demarcated and to have an eccentric juxtacortical position, although in 1 instance (involvement of a metatarsal bone) it did come to expand and replace virtually the entire bone.

On the clinical side it may be noted that most of the patients were adolescents or young adults, though some were older. The lesion, as a rule, evolves slowly and is often of some months' or even a few years' standing before surgical intervention is sought. The roentgenographic picture has a certain distinctiveness, at least when the lesion is in a long bone and has attained appreciable size, although there may be difficulty at times in differentiating it from bone cyst, enchondroma or a focus of fibrous dysplasia, without examination of tissue. The tumor is apparently entirely benign and does not tend to recur after curettage, even without supplementary irradiation. While the tumor is not a particularly common one, its recognition is of some importance in that pathologically it may readily be mistaken for sarcoma and, as such, treated more radically than is necessary.

GENERALIZED OCHRONOSIS

Report of an Instance in Which it Was Misdiagnosed as Melanosarcoma, with Resultant Enucleation of an Eye

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CHICAGO

IN A COMPREHENSIVE REVIEW of ochronosis of the sclera and cornea published in 1942, Smith[1] stated that up to the time of his report ocular ochronosis had never been described in an American textbook of ophthalmology. It is possibly for this reason that the misdiagnosis to be described, with its tragic sequel, occurred.

REPORT OF CASE

M. A., a 68 year old white man, resident in a large charity institution maintained for destitute citizens, was largely lost track of medically in the midst of his fellow residents, and only a scanty history is available. When first admitted to the institution, in 1926, he stated that thirty-one years earlier, when he was 21, his right foot had been partially cut off, and that five years before his admission he had begun to have rheumatism. He further stated that his left eye had been lost traumatically some time previously. On examination a systolic murmur was noted at the cardiac apex. In 1927 a diagnosis of diabetes mellitus was entertained. From 1939 till his demise he was seen repeatedly in the institution's infirmary. He complained of marked pain in many of his joints. On some occasions the pain was so incapacitating that he was unable to walk. A diagnosis of osteoarthritis was made. Two analyses of urine, in 1939 and 1940, were strongly positive for sugar. A determination of blood sugar performed in association with the latter examination of urine yielded 80 mg. per hundred cubic centimeters. A questionable diagnosis of diabetes continued to be entertained. Three subsquent urinalyses, in 1941, revealed no sugar.

In March 1941 the patient complained of pain in his right eye. The eye showed blue-gray discoloration of the sclera, more marked laterally than nasally. Examinations of blood and urine showed nothing significant, and on April 10, 1941 the right eye was enucleated because of a diagnosis of melanosarcoma. There was no tumor extension beyond the eyeball. Postoperatively the enucleated eye revealed bluish gray discoloration of the sclera, but no tumor mass was found, and the diagnosis of melanosarcoma was questioned. The eye, fixed in formaldehyde solution U.S.P., was sent to another institution for pathologic examination. A gross examination only was made. The sclera was found to be darkly pigmented, but no neoplastic growth or other abnormality was noted. The discoloration was

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1. Smith, J. A.: J. A. M. A. **120**:1282, 1942.

thought to be due to improper fixation or to fixation artefact, and the specimen was disposed of.

One year later, in March 1942, the patient displayed edema of both legs, and a diagnosis of arteriosclerotic heart disease with heart failure was made. The urine was red (on previous occasions it had been described as "straw colored," and "amber"). It contained many pus cells and gave a strongly positive reaction for albumin but none for sugar. His temperature rose to 102.1F. and remained at about

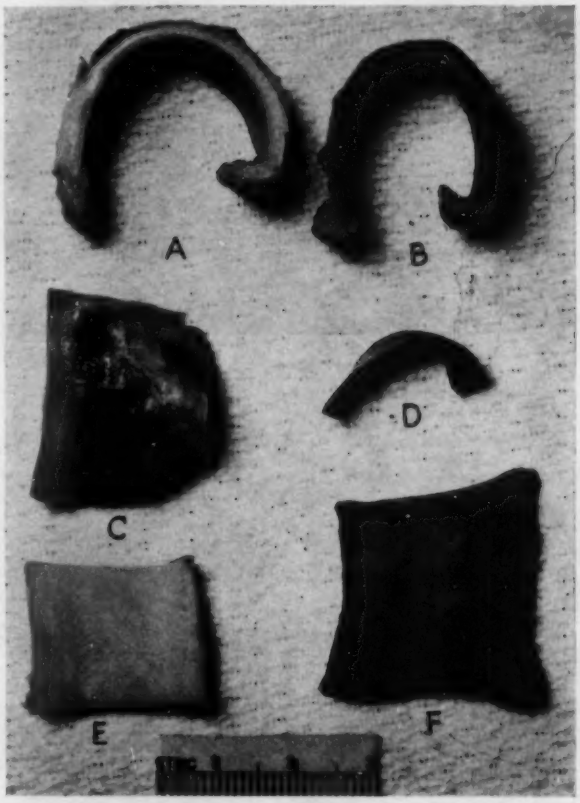

Fig. 1.—*A*, cross section of a normal trachea; *B*, cross section of an ochronotic trachea; *C*, ochronotic aorta; *D*, cross section of an ochronotic aorta; *E*, normal aorta; *F*, ochronotic aorta.

this level till he died March 30, 1942, with marked respiratory difficulty and cyanosis, interpreted as being signs of bronchopneumonia.

Necropsy (ten day post mortem).—Post mortem changes were advanced in many tissues.

The moderately well nourished body, weighing an estimated 155 pounds (70 Kg.) and measuring 67 inches (170 cm.) in length, was deformed by marked dorsal kyphosis and mutilated by old, healed amputation of the right foot in the midmetatarsal region. Both eyes were enucleated, and the orbital cavities were largely filled with grayish white fibrous tissue. Gross and microscopic examination

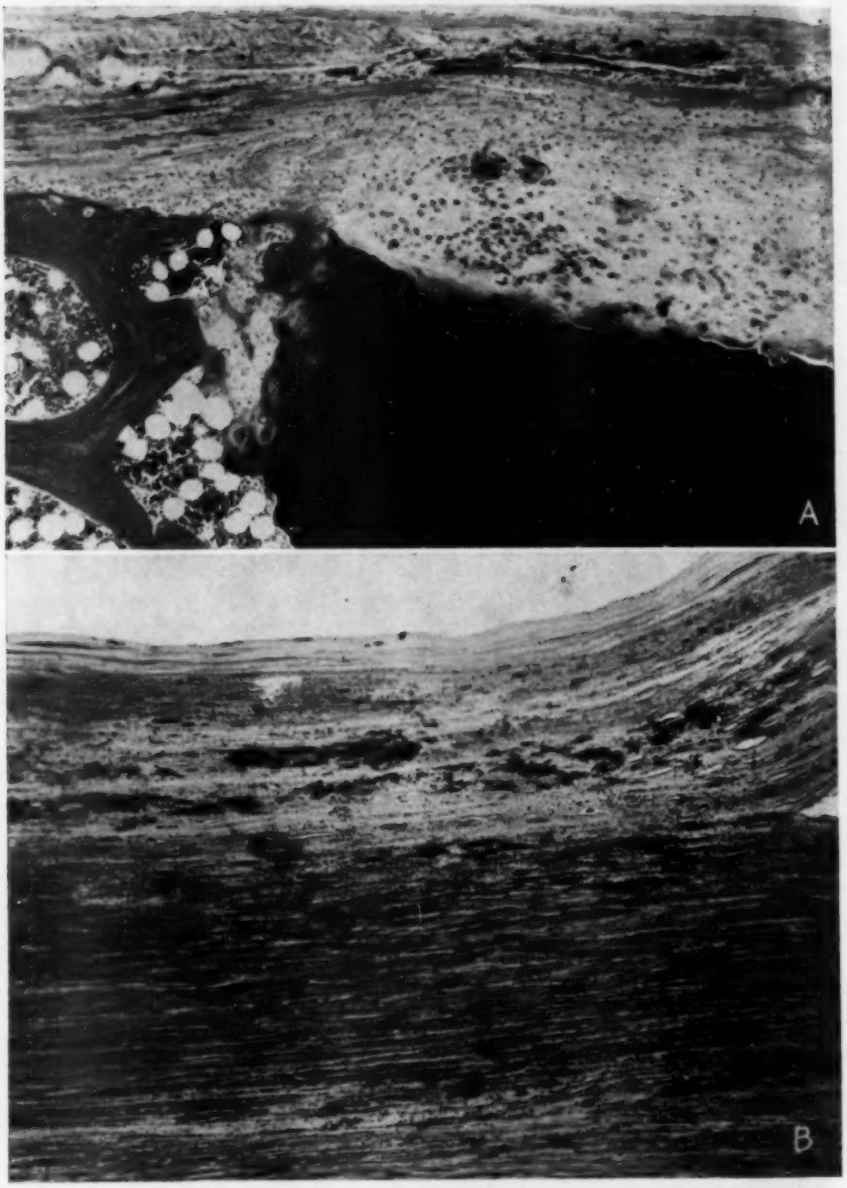

Fig. 2.—*A*, ochronotic pigmentation of costal cartilage; hematoxylin-eosin stain; × 75. The lack of pigmentation of the outer callous cartilage, presumably formed because of instability at the illustrated costochondral junction, may be due to the active functioning and recent formation of this cartilage.

B, ochronotic pigmentation of the aorta; hematoxylin-phloxine stain; × 115. The pigment is most prominent in areas of degenerative change.

of this tissue failed to reveal any evidence of neoplastic growth. The ears had a bluish discoloration, as did also the fingernail beds, but this was partially masked by the generalized cyanosis of the head, neck and upper extremities.

A most striking and unusual finding was the raven black pigmentation of all cartilages examined. The costal cartilages, the tracheal cartilaginous rings, and the remnants of the intervertebral disks were all completely pigmented (fig. 1). Wherever there was overlying mucosa (e.g., in the trachea and the bronchi), it assumed a grayish discoloration because of this black pigmentation of the underlying cartilage. Similar but less intense pigmentation was found in the endocardium and the aortic endothelium. It was more intense about the scarred and calcified areas of the cardiac valves. Wherever there were accumulations of atheromatous and calcified material, the pigmentation varied from gray to black (fig. 1). It was evident also in other scar tissues, such as the thickened splenic capsule and the apical pulmonary scars. Microscopically, the pigment did not take any of the usual stains employed and did not stain for iron. By contrast it was best brought out with Masson's trichrome stain. In the cartilage the pigment appeared light yellow to dark brownish black, depending on the amount present. Here it was homogeneous and not evidently particulate (fig. 2*A*). About the cartilages, in the periosteum, in the aorta, and in the scarred areas of the spleen and heart it varied slightly in form. Where only a small amount of pigment was present it tended to be fine and diffuse, and definite particulate form could not be made out microscopically. Where it was denser, it was more particulate, though in cartilage it stained the cartilaginous matrix diffusely. Where slight in amount, it tended to be lighter yellow than the golden brown of hemosiderin. Lying in connective tissues it stained collagenous fibers yellow, but where particulate it was often contained in macrophages, though at times it was also free in the tissues. Prostatic calculi also were stained.

The right pleural cavity contained 1.5 liters of fibrin-flecked fluid, and the right lower pulmonary lobe was atalectatic. Foci of hypostatic bronchopneumonia and generalized pulmonary edema were evident both grossly and microscopically. The left lung weighed 720 Gm. and the right 500 Gm.

The emptied heart, together with the ascending aorta, weighed 700 Gm. The right myocardium averaged 8mm. and the left 15 mm. in thickness. The leaflets of the aortic valve were rigid, a condition due to fibrosis and calcification. The leaflets of the mitral value were likewise markedly fibrotic and calcified, and the chordae tendineae were slightly thickened. The tips of the papillary muscles were fibrotic and calcified. Ochronotic pigmentation was evident in all these scarred foci. The aorta showed severe calcific arteriosclerotic changes, but the coronary arteries were relatively free of such degenerative changes, as were also the pulmonary arteries. Microscopically the walls of the pulmonary arterioles were, however, thickened.

In addition to these findings the anatomic diagnosis included arthritic fusion and deformity of the vertebrae, osteoporosis, passive congestion of lungs, liver and spleen, minimal focal chronic pyelonephritic scarring, moderate arteriolonephrosclerosis, fibrous epislenitis and marked atrophy of the right testis. The pancreas gave no evidence of diabetic hyalinization of the islets.

COMMENT

Ochronosis has been found to be associated with a defect of the metabolism of tyrosine and phenylalanine, characterized by a failure in the further breakdown of homogentisic acid, one of the interme-

diates of this metabolic pathway. Little is known concerning the composition of the pigments deposited in ochronosis, though they are generally thought to resemble melanin. It is probable that their composition varies with the underlying cause of their formation[2]. The concept of the mode of production of the pigment advanced by Pick[3] is still the most plausible. He suggested that the phenol substances of exogenous ochronosis and the homogentisic acid appearing in the endogenous group are changed into melanin by the action of the oxidative ferment tyrosinase. The pigment has been found in congenital inborn errors of metabolism, in association with melanuria in perverted melanin metabolism, and in the exogenous type, with long-continued external use of phenol U.S.P. and other phenols in dressings and bandages. The latter group is especially well discussed by Fishberg[4].

It is generally recognized, as stated by Howard and Mills[5], that the triad of symptoms including pigmentation of the sclera and ears, dark color of the urine and arthritis is pathognomonic of ochronosis. Homogentisic acid, excreted in the urine and present in perspiration, has the property of turning black when oxidized, and this accounts for the dark color that develops in the urine of persons with alkaptonuria on standing and for the dark staining of clothes by the perspiration of these persons. In the instance here reported, the clinical history is too scanty to determine whether the "red urine" reported on one occasion was that of alkaptonuria or whether it was red from some other cause. The lack of pigmentation recorded at other times may have been due to the urine's having been examined before sufficient time had elapsed to permit the pigment to form. The small amount of urine in the bladder at necropsy was not abnormally pigmented. The pigmentation of the ears, as well as of other tissues of the body, especially the cartilages, together with the arthritic changes of the larger joints and of the vertebrae, leaves little doubt that this represents a true instance of ochronosis. The fact that diabetes was diagnosed on the basis of the reducing power of the patient's urine bears out the diagnosis of ochronosis, especially in the presence of the normal level of blood sugar determined, since homogentisic acid is known to reduce the alkaline copper solutions (Benedict's and Feh-

2. Bodansky, M., and Bodansky, O.: Biochemistry of Disease, New York, The Macmillan Company, 1940, p. 570.

3. Pick, L.: Berl. klin. Wchnscher, **43**:478, 1906.

4. Fishberg, E. H.: Virchows Arch. f. path. Anat. **251**:376, 1924.

5. Howard, C. P., and Mills, E. S.: Oxford Medicine, New York, Oxford University Press, 1941, vol. 4, pt. 1, p. 223.

ling's) and may thus, by suggesting glycosuria, lead to an error of diagnosis[6].

Beddard[7] has called attention to the frequent association of ochronosis and cardiovascular lesions, and it has been suggested that these changes are also primarily dependent on the metabolic disorder. In the case reported here, the distribution of the lesions was similar to that usually seen in rheumatic fever, but the histologic characteristics were not clearcut for this disease, and the true cause of the calcific valvulitis remains undetermined.

It seems reasonable to assume that the pigmentary changes found in the eye and diagnosed as melanosarcoma actually represented ochronotic pigmentation, especially in view of the fact that no neoplasm was found in the postoperative examination of the eye and since no residual neoplastic tissue was found at necropsy. It was extremely unfortunate that the patient had previously lost his other eye traumatically, since the operation thus left him totally blind.

No similar instance of misdiagnosis with enucleation of the eye has been found in the literature, and none is mentioned by Smith[1], who carefully reviewed all papers on ochronosis with particular reference to involvement of the eye. Instances have, however, probably occurred in which such a diagnosis has been entertained but not mentioned when the final diagnosis was correctly established and reported. Not a few instances have been reported in which the diagnosis of ochronosis was not established till necropsy. Poulsen[8], who was fortunate enough to observe 9 instances of this disorder, reported that in the case of the patient designated Anna Magdaline B. a diagnosis of melanosarcoma was entertained. The knowledge that the predominant origin of this neoplasm is in the choroid, when it occurs in the eye, and that the sclera is involved only secondarily, should sugegst caution in diagnosing melanosarcoma of the eye on the basis of pigmentary discoloration of the sclera. The presence of the other pathognomonic signs of ochronosis should make the diagnostician extremely suspicious of a diagnosis of this neoplasm.

Schreiber[9] stated that arsenic melanosis, diffuse toxic goiter and Addison's disease must be considered in the differential diagnosis of

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6. Duncan, G. G.: **Disease of Metabolism**, Philadelphia, W. B. Saunders Company, 1942, p. 600.

7. Beddard, A. P.: Quart, J. Med. **3**:329, 1909.

8. Poulsen, V.: **Om ochronotiske tilstande hos mennesker og dyr**, Copenhagen, Jacob Lunds Medical Bookstore, 1910; translated, Beitr. z. path. Anat. u. z. allg. Path. **48**:346 and 437, 1910.

9. Schreiber, L.: **Handbuch der gesamten Augenheilkunde**, ed. **3**, Leipzig, Wilhelm Englmann, 1924, p. 183.

ochronotic pigmentation. In this light it is interesting to note that the first British case of ochronosis was first diagnosed as a case of Addison's disease[10]. Smith[1] suggested that melanosis bulbi and pigmentary senile degeneration of the sclera should likewise be considered in the differential diagnosis.

SUMMARY

A report is made of an instance of enucleation of the remaining eye of a patient suffering from ochronosis, whose other eye had previously been lost traumatically. The enucleation was performed because of a misdiagnosis of the ocular ochronotic pigmentation as melanosarcoma. The necropsy observations are described, and the differential diagnosis is briefly discussed.

10. Pope, F. M.: Lancet 1:24, 1906. Smith.[1]

MODIFIED PHOSPHOTUNGSTIC ACID-HEMATOXYLIN STAIN

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DESPITE the number of mordants proposed, it is difficult to make consistently good phosphotungstic acid-hematoxylin stains on tissues fixed in formaldehyde solution. This difficulty may be due to an inadequately oxidized staining solution. In our hands, potassium permanganate and hydrogen peroxide have not proved to be good oxidants, and not even the supposedly reliable method of "spontaneous" ripening has given sharply defined stains. Finally the following method of oxidation was tried, which has been completely successful in every detail.

PREPARATION OF STAINING SOLUTION

Phosphotungstic acid	10 Gm.
Hematoxylin	500 mg.
Red mercuric oxide	250-500 mg.
Hydrogen peroxide	2 cc.
Distilled water	500 cc.

Dissolve the hematoxylin in a little of the water with the aid of heat. In the meantime, dissolve the phosphotungstic acid in the rest of the water with the aid of heat and then add the hematoxylin solution. Bring this mixture to a boil. Cautiously add the mercuric oxide, remove the vessel from the flame, shake it to put any undissolved oxide into solution and then set it aside to cool. When the solution is somewhat cool, add the hydrogen peroxide. Cover lightly and wait several days to a week before using. The solution should assume a deep brownish red color. It can be used repeatedly.

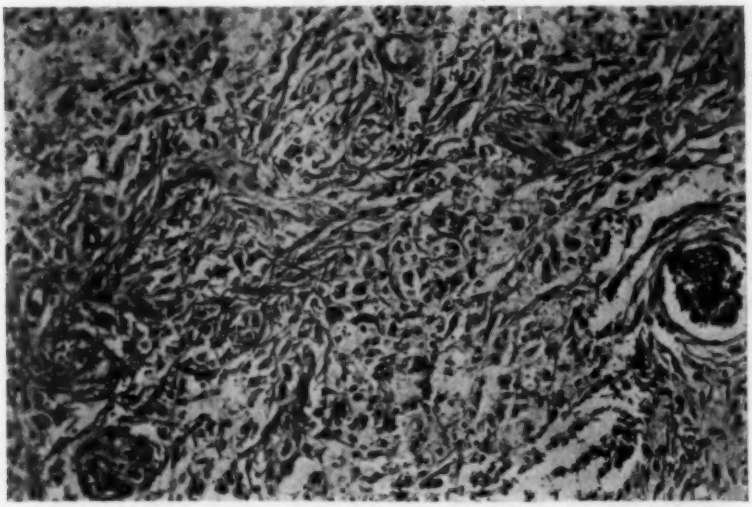
METHOD OF STAINING

1. Bring deparaffinized sections of formaldehyde-fixed material down to water.
2. Place for five minutes in 0.5 per cent potassium permanganate (freshly prepared from a 5 per cent solution).
3. Rinse in tap water.
4. Bleach in 2 per cent oxalic acid for five minutes.
5. Wash in tap water and then in distilled water.
6. Mordant for one hour in 4 per cent ferric alum.
7. Rinse in tap water and then in distilled water.
8. Place in staining solution for two to twenty-four hours.

From the Institute of Pathology, Western Reserve University.

9. Dehydrate in two changes of absolute isopropyl alcohol, clear in xylene and mount.

Even though formaldehyde-fixed material is being used, the bleaching in potassium permanganate and oxalic acid is necessary to give sharp contrast.



Section of glioblastoma multiforme of the brain to show the sharply stained fibrils.

The mordanting in the ferric alum intensifies the color of the blue-staining elements.

The time in the staining solution should be watched, because the solution acts so quickly that in only rare instances is it necessary to wait for overnight action. Staining is usually complete in two to three hours.

Notes and News

Appointments, Etc.—The board of scientific directors of the Rockefeller Institute for Medical Research announces the promotion of Alfred E. Mirsky and D. Wayne Wooley from associate member to member and the appointment of R. M. Archibald as member. Maclyn McCarty has been promoted from associate to associate member. New appointments include: associate—Dominic Dziewiathowski; assistants—Margaret Oakley Dayhoff, Jacques Genest, Herbert Jaffe, Edwin D. Kilbourne, George E. Palade, Stanfield Rogers, Chandler A. Stetson Jr., Harrison F. Wood, and R. Barclay McGhee. The board also announces that D. D. Van Slyke, who has reached the age of retirement, has been made member emeritus of the institute.

C. A. Krakower has been promoted to professor of pathology at the University of Illinois College of Medicine. He also will serve as associate pathologist in the University's 428-bed research and educational hospitals. He has been a member of the faculty of the University since 1944. He previously served on the staff of Tulane University Medical School, Columbia University School of Tropical Medicine, and Harvard Medical School. Born in Montreal, Canada, he received his Bachelor of Science and Doctor of Medicine degrees from McGill University. In the past he has been particularly interested in tropical diseases. His research activities now concern cardiovascular diseases.

H. H. Catchpole has been promoted to assistant professor of pathology in the University of Illinois College of Medicine. Dr. Catchpole has conducted extensive work in the field of physiology, with special reference to endocrine problems. While serving with the Navy during the war, he investigated problems of decompression, chemical oxygen systems and nutrition. He has been a member of the University of Illinois faculty since 1946.

Death.—R. S. Austin, professor of pathology at the University of Cincinnati College of Medicine, died, April 30, 1948, from carcinoma of the pancreas, aged 63 years.

Grants and Fellowships of the American Cancer Society.—The committee on growth of the Society is entertaining applications for grants and fellowships. Applications for extension of existing grants in cancer research will be received until October 1; applications for new grants, until November 1. Final decision on applications submitted during this period will be made in most cases soon after February 1. Grants approved at this time ordinarily will become effective on July 1, 1949. Fellowship applications may be submitted at any time. Those received prior to November 1 will be acted on by the committee in December. Those received between November 1 and March 1 will be acted on in April. Fellowships ordinarily will begin on July 1, though this date may be varied at the request of the applicant. During the past year the American Cancer Society, Inc., has approved research grants and fellowships totaling over $2,000,000. Communications regarding grants and fellowships should be addressed to Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

Society News.—The Human Genetics Society of America has been organized with about 200 members. The president is H. J. Muller, the vice president L. H. Snyder, the secretary Herluf H. Strandskov, University of Chicago. The first meeting will be held in Washington, D. C., September 11 to 13 next.

The second Interamerican Congress on Brucellosis will be held in Argentina in the latter part of November 1948. Correspondence about the Congress should be sent to Dr. Alice C. Evans, president, 2001 Connecticut Ave., N. W., Washington 8, D. C.

Dr. Eileen Mullin, formerly resident in roentgenology in the Little Company of Mary Hospital, has joined the staff of the Chicago Tumor Institute.

Books Received

IDENTIFICATION OF TUMORS: ESSENTIAL GROSS AND MICROSCOPIC PATHOLOGIC FEATURES SYSTEMATICALLY ARRANGED FOR EASIER IDENTIFICATION. By N. Chandler Foot, M.D., professor of surgical pathology, Cornell University Medical College and surgical pathologist to New York Hospital. Pp. XXI and 397, with 241 illustrations. Price $6. Philadelphia; London, England; Montreal, Canada: J. B. Lippincott Company, 1948.

This small volume by an eminent author and surgical pathologist is aptly described by its subtitle. It is a short guide to tumor diagnosis, larger but comparable to those available for identification of birds and flowers. It is not designed to replace textbooks but to aid students and graduates in quick identifications. Following a preface, but no general or introductory chapters, the text immediately covers specific tumors in twenty-one chapters. The book is subdivided into two parts, one of which takes up neoplasms of general distribution and the other those of specific systems and organs. The logic of this organization is not apparent, since the first section includes chapters on organs and systems, such as the spleen, the skeletal system, the cardiovascular system and the serous membranes, while the latter part contains the nervous system, whose anatomic ramifications and tumors are fully as general as any of those named. In each chapter benign tumors and their cancerous analogs are briefly characterized as to source, site, age and sex of patient, gross appearance, microscopic appearance, variants, metastasis and prognosis.

The style varies from staccato through telegraphic to fluent. The descriptions are brief, clear and, on the whole, accurate. Because they are of necessity so concise, some appear arbitrary and dogmatic. A few might be challenged as unreliable at best and inaccurate at worst. For example, the characterizing of acute and chronic myelogenous leukemia as a disease of young adults, and one whose masses in the acute form . . . "are often of a jade-green . . . known as 'chloromas' " would not have wide approval. Neither should the statement that pulmonary adenomas are parenchymal tumors not associated with bronchi, or the statement that gallbladder tumors are extremely rare, be accepted as accurate.

The author states that this is not a synopsis of oncology, but it will probably be adopted and used as such. A need may exist for this type of book, but it is regretted that present teaching is such that it does. It is hoped that no one is making important decisions in the diagnosis and treatment of tumors whose knowledge is not advanced far beyond the level which could be benefited by this guide. For those at an admittedly earlier stage of development this book should be useful. The diagnosis of cancer is more difficult than the identification of birds and flowers but no less interesting, and much more important.

APPROACHES TO TUMOR CHEMOTHERAPY: A SYMPOSIUM OF PAPERS AND DISCUSSIONS ON VARIOUS ASPECTS OF TUMOR CHEMOTHERAPY, DEVELOPED FROM THE SUMMER MEETINGS OF THE SECTION ON CHEMISTRY (C) OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE AT GIBSON ISLAND, MARYLAND, 1945-1946. Edited by Forest Ray Moulton. Washington, D. C.: American Association for the Advancement of Science, 1947.

This volume, whose orignns and content are described in the subtitle, summarizes much recent research concerning the chemotherapy of tumors. The numerous papers contributed by nearly one hundred authors are grouped into sections on special methodology, nutritional factors, bacterial products, nitrogen mustards, and miscellaneous subjects, chiefly clinical. It is impossible to review here each paper separately. Together they afford an excellent survey of this rejuvenated and important line of research. Most of the papers summarize many years of effort. Excellent bibliographies and valuable discussions follow nearly every chapter. This monograph is highly recommended to all pathologists and cancer research workers.